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Vascular Flora of Bonnie's Prairie Nature Preserve, Iroquois County, Illinois

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ABSTRACT

Bonnie's Prairie Nature Preserve, located in Iroquois County, Illinois, occurs in the Kankakee Sand Area Section of the Grand Prairie Division. Dominant plant communities within this 4.3 ha preserve include dry sand prairie, temporary sand pond and wet-mesic sand prairie. During the growing seasons of 2005 and 2006, dominant community types within the preserve were sampled and a vouchered inventory of the preserve was conducted. A total of 248 taxa were found within the preserve: 5 ferns and fern allies, 77 monocots, and 166 dicots. Plant families most highly represented included the grass family (Poaceae) with 38 taxa, sunflower family (Asteraceae) with 25 taxa, sedge family (Cyperaceae) with 18 taxa, and rose family (Rosaceae) with 17 taxa. Thirty taxa (12.1% of the flora) were adventive to the site and the Floristic Quality Index (FQI) (with adventives) was 51.1. Dominant species in the dry sand prairie were *Schizachyrium scoparium* (little bluestem), *Achillea millefolium* (yarrow), fall witch grass *Leptoloma cognatum* (fall witch grass), and *Poa pratensis* (Kentucky blue grass). Dominant species of the sand pond were *Glyceria septentrionalis* (floating manna grass), *Persicaria coccinea* (scarlet smartweed), *Bidens cernua* (nodding bur marigold), and *Sparganium androcladum* (branched bur-reed). The wet-mesic sand prairie was dominated by *Calamagrostis canadensis* (blue joint grass), followed by *Persicaria coccinea*, *Erechtites hieracifolia* (fireweed), and *Bidens coronata* (tall swamp marigold). No threatened or endangered species were located during the surveys.

INTRODUCTION

At the turn of the nineteenth century, tallgrass prairie communities occupied approximately 55 – 60% of the total land area in Illinois (7.9 – 8.7 million ha) (Vestal 1931, Anderson 1970, Iverson 1988, Szafoni et al. 2002). Between the years of 1820 and 1980, approximately 99.99% of these tallgrass prairie communities were lost due to agriculture, urban development and succession (White 1978, Iverson 1988). Today, tallgrass prairie communities east of the Missouri River are considered critically endangered ecosystems, now occupying less than 2% of their former range (Christensen et al. 1996).

Tallgrass prairie community subclasses in Illinois include gravel prairie, dolomite prairie, hill prairie, shrub prairie, and sand prairie, with sand prairies representing the largest extant prairie remnants (White 1978, White and Madany 1978). Extensive sand deposits within the state supporting sand prairie and associated sand communities include: 1) the Chicago Lake Plain Section and the Lake Michigan Dunes Section of the Northeastern

Morainal Natural Division, 2) the Green River Lowland Section and the Kankakee Sand Area Section of the Grand Prairie Natural Division, and 3) the Illinois River Section and Mississippi River Section of the Illinois River and Mississippi River Sand Areas Natural Division (Hart and Gleason 1907, Gleason 1910, Schwegman 1973, Lineback 1979, Swink and Wilhelm 1994).

At the time of settlement, sand deposits and resulting sand prairie and associated sand communities covered approximately 497,248 ha (3.4 %) of the state (Lineback 1979, Fehrenbacher et al. 1984). Several early authors studied and described many of the sand communities in various regions (Higley and Raddin 1891, Cowls 1899, McDonald 1900, Hart and Gleason 1907, Gleason 1909, 1910, Gates 1910, 1912, Fell 1957), and these studies have contributed greatly to our present understanding of the historic structure and composition of these areas. In the following decades, however, research subsequent to these initial studies was generally lacking. With the invention of central pivot irrigation in the mid 1900s, many of Illinois' sand regions were rapidly converted to agriculture. In the late 1970s, it was ascertained that less than 0.5% of the state's native sand prairie and associated sand communities still existed in a relatively undisturbed condition (White 1978). Today, in light of such extensive losses, comprehensive studies of our extant remnants are imperative. The present study was undertaken to determine the vascular plant species composition, community structure, and floristic integrity of sand communities occurring in Bonnie's Prairie Nature Preserve (BPNP).

STUDY SITE

Located in Iroquois County, Illinois, approximately 3.5 miles north of Watseka (NE ¼ NW ¼ NE ¼ S17 T27N R12W), BPNP occurs in the Kankakee Sand Area Section of the Grand Prairie Natural Division (Schwegman 1973). This area is characterized by extensive sand formations that were deposited approximately 14,000 to 16,000 years ago by glacial meltwater activity and were subsequently worked and reworked into dune and sheet-like formations by strong winds (Willman and Frye 1970, Wiggers 1997, Killey 1998). Additionally, the deposits at BPNP occur in an area that was once the lakebed of the ancient glacial Lake Watseka, which had drained hundreds of years previous to the deposition of these sands (Willman and Frye 1970, Frankie et al. 1996, Wiggers 1997, Killey 1998, Follmer 2006).

BPNP was dedicated as a state nature preserve in 1992, and is 4.3 ha (10.6 acres) in area. Dominant plant communities in the preserve include dry sand prairie, temporary sand pond, and wet-mesic sand prairie. Smaller and/or more non-contiguous communities in the preserve were characterized by dry sand savanna, degraded dry-mesic sand prairie, marsh/wet sand prairie, and scrubland areas. Environmental heterogeneity resulted in combinations of these community types intergrading almost imperceptibly in many parts of the preserve.

Surficial deposits within the preserve are of the Henry Formation and consist of glacial outwash dominated by sand and gravel and are classified as Parkland facies, which consist of windblown sands in dunes or sheet-like deposits (Willman and Frye 1970, Hansel and Johnson 1996). Soil types as described by Kiefer (1982) indicate the dry sand prairie and dry sand savanna areas occur in excessively drained Chelsea fine sand, while the dry-

mesic sand prairie area occurs on somewhat poorly drained Orthents, loamy soils, which are characterized by surface layers of fine sandy loam or loamy fine sand. The wet-mesic sand prairie areas within the preserve occur on the more poorly drained Roby loamy fine sand. The sand pond areas occupy a position on the landscape where the Chelsea fine sand and Roby loamy sand converge.

East-central Illinois is characterized by a continental climate, having hot summers and cold winters (Fehrenbacher et al. 1984). In the vicinity of BPNP, the mean annual temperature as reported by the Midwestern Regional Climate Center from historical climate data at Watseka, IL, collected between 1971 and 2000, is 50.0° F (10.0° C) (MRCC 2006). July and August are the warmest months, with mean temperatures of 23.3° C (73.9° F) and 22.1° C (71.7° F), respectively, and January and February are the coldest, with mean temperatures of –5.5° C (22.1° F) and –2.8° C (26.9° F), respectively (MRCC 2006). The mean annual precipitation is 91.4 – 96.5 cm (36 – 38 in.), with the highest levels of precipitation occurring during the month of June [11.7 cm (4.62 in.)] (Fehrenbacher et al. 1984, MRCC 2006). The mean number of frost-free days in this region ranges from 160 to 170 (Fehrenbacher et al. 1984).

MATERIALS AND METHODS

During the growing seasons of 2005 and 2006, multiple visits were made to BPNP to inventory and sample the vegetation. Voucher specimens were collected for all vascular plant taxa occurring within the preserve, and habitat data and GPS coordinates were recorded for all collections. Collections were identified and deposited in the herbarium of the Illinois Natural History Survey (ILLS), Champaign, Illinois. Nomenclature follows Mohlenbrock (2002).

Ground flora compositions were evaluated by placing 1.0 m^2 quadrats at every other meter (i.e., 0, 2, 4, etc.) along 100m line transects (50 m in sand pond) in the largest and highest quality representative areas for dominant community types (Figure 1), as well as the marsh/wet sand prairie pond margin between sand pond and wet-mesic sand prairie. Quadrats were placed in an alternating pattern along transects, with every other quadrat being placed to the right or left. Cover values of all species rooted within quadrats were estimated using Daubenmire (1959) cover classes as modified by Bailey and Poulton (1968), and are as follows: class 1 = 0 – <1%, class 2 = 1 – <5%, class 3 = 5 – <25%, class 4 = 25 – <50%, class 5 = 50 – <75%, class 6 = 75 – <95%, and class 7 = 95 – 100%. From these data, frequency, relative frequency, mean cover, relative cover, and importance value (sum of relative frequency and relative cover) were calculated for each species.

To further evaluate floristic integrity, the mean coefficient of conservatism ($\overline{C}$) and floristic quality index (FQI = [I]) were calculated for the entire preserve as well as the dominant community types, according to Taft et al. (1997), using the following formulae, respectively: $\overline{C} = \sum C/N$, where C is the coefficient of conservatism and N is the number of taxa; and $I = \overline{C}\ (\sqrt{N})$, where I is a weighted index of species richness, and is the product of $\overline{C}$ multiplied by the square root of the number of species ($\sqrt{N}$).
Coefficients of conservatism (C) assigned to all vascular plant taxa occurring within the state, according to Taft et al. (1997), consist of a value ranging from 0 to 10 and represent

a measure of each taxon's tolerance to habitat degradation. A *C*-value of 10 indicates the highest degree of fidelity to high quality natural areas, while a value of 0 indicates the lowest. Following this, taxa at the upper end of the conservatism spectrum (i.e., 7-10) are usually the first species to disappear as natural areas undergo various types of disturbance that lead to habitat degradation. Non-native taxa are automatically assigned a *C*-value of 0. For areas intensively surveyed, the FQI provides a rapid and effective means for making qualitative comparisons of floristic integrity among sites. Sites with a FQI (I) ≥ 35 or $\overline{C}$ -value ≥ 3.5 are considered regionally noteworthy – possessing sufficient floristic quality to be considered at least marginally high quality natural areas (Swink and Wilhelm 1994, Taft et al. 1997).

Lastly, historic aerial photographs were obtained from the University of Illinois Map and Geography Library, and were examined for each decade beginning with 1940 to further assess past conditions of the site.

RESULTS AND DISCUSSION

Vascular Plant Species Present

A total of 248 species representing 69 families and 168 genera where documented at BPNP (Appendix 1). Of these taxa, 30 (12.1%) were adventive to the site and eighteen (60.0%) of the adventive taxa occurred in, but were not restricted to, the dry sand prairie community in the northeast portion of the preserve. The majority of the remaining adventive taxa occurred in scrubland areas and degraded edges of the preserve (Figure 1). Pteridophytes accounted for 5 taxa, in 4 genera, and 4 families. Among angiosperms, monocots accounted for 77 taxa, in 46 genera, and 11 families, and dicots, 163 taxa, in 116 genera, and 54 families. The Poaceae and Asteraceae represented slightly over 25% of the flora at BPNP, with 38 taxa (15.3%) and 25 taxa (10.1%), respectively, followed by the Cyperaceae [18 taxa (7.3%)], Rosaceae [17 taxa (6.9%)], Fabaceae [12 taxa (4.8%)], and Polygonaceae [11 taxa (4.4%)]. With respect to physiognomy, forbs accounted for 59.2% of the flora, grasses and sedges 22.6%, shrubs 8.5%, trees 5.6%, vines 2.0%, and ferns/fern allies 2.0%. The native FQI for the entire preserve was 54.5 (51.1 with adventive taxa) and the native $\overline{C}$ -value was 3.7 (3.2 with adventive taxa), indicating a natural area still possessing a high degree of natural integrity.

Dry Sand Prairie

The dry sand prairie community, along with a small inclusion of dry sand savanna occurring in the northeast portion of the preserve (Figure 1 – areas 1A & 7A) was moderately degraded, but still possessed a reasonable degree of floristic integrity. The small inclusion of dry sand savanna appears on historical aerial photographs to have been connected to a larger sand savanna just north of the preserve. Woody encroachment is advancing in this sand savanna inclusion as well as on all sides of the sand prairie. The western and southwestern edges of this area, where the dry sand prairie begins to transition to the northernmost sand pond, are being heavily invaded by *Rubus allegheniensis* Porter (common blackberry). Woody species encroaching on the north, east and southeast boundaries of this area include *Crataegus crusgalli* L. (cockspur hawthorn), *Lonicera maackii* (Rupr.) Maxim. (amur honeysuckle), *Morus alba* L. (white mulberry), *Prunus serotina* Ehrh. (black cherry), *Quercus velutina* Lam. (black oak), *Rhus glabra* L. (smooth sumac), *Rosa multiflora* Thumb. (multiflora rose), *Sassafras albidum* (Nutt.) Nees (sassafras), *Toxi-*

codendron radicans (L.) Kuntze (poison ivy), and *Vitis riparia* Michx. (riverbank grape). Several taxa confined to these more shaded successional areas included: *Asplenium platyneuron* (L.) Oakes (ebony spleenwort), *Galium circaezans* Michx. var. *hypomalacum* Fern. (wild licorice), *Polygonatum commutatum* (Schult.) A. Dietr. (Soloman's seal), *Sanicula canadensis* L. var. *canadensis* (black snakeroot), and *Smilacina stellata* (L.) Desf. (starry false Soloman's seal).

A total of 101 species were encountered in the approximately .26 ha (.64 acre) dry sand prairie and 42 taxa occurred within the sampling quadrats (Table 1). The dominant species was *Schizachyrium scoparium* (Michx.) Nash (little bluestem) with an Importance Value (IV 200%) of 36.6%. Other important taxa included three adventives *Achillea millefolium* L. (yarrow) (IV 26.6%), *Poa pratensis* L. (Kentucky bluegrass) (IV 16.0%), and *Rumex acetosella* L. (field sorrel) (IV 11.1%); and the natives, *Leptoloma cognatum* (Schult.) Chase (fall witch grass) (IV 20.0%), *Rubus flagellaris* Willd. (common dewberry) (IV 13.2%), and *Phlox bifida* Beck. (cleft phlox) (IV 12.9%) (Table 1). Although only five adventive taxa occurred within sampling quadrats (Table 1), the high importance values of three of these taxa are indicative of the more degraded condition of this remnant prairie and reflect a land use history which likely included cattle grazing. Additionally, the dry sand prairie community had more adventive taxa than any other community type in the preserve, with 13 additional adventives occurring here (Appendix 1). The majority of the more conservative taxa including, *Amorpha canescens* Pursh (leadplant), *Anemone cylindrica* Gray (thimbleweed), *Asclepias amplexicaulis* Small (sand milkweed), *Dalea purpurea* Vent. (purple prairie clover), *Helianthemum canadense* (L.) Michx. (common rockrose), *Helianthus occidentalis* Riddell (western sunflower), *Lechea mucronata* Raf. (hairy pinweed), *Liatris aspera* Michx. (rough blazing star), *Sporobolus heterolepis* (Gray) Gray (northern drop seed), and *Tephrosia virginiana* (L.) Pers. (goat's rue), were more scattered and/or infrequent in this community type. The native FQI for this area was 31.6 (28.7 with adventive taxa) and the native $\bar{C}$-value was 3.5 (2.9 with adventive taxa). These values support the interpretation of a moderately degraded habitat, but one that still possess a noteworthy assemblage of plants.

Sand Pond

The northernmost sand pond at BPNP is the deeper and more diverse of the two sand ponds occurring within the preserve and the only of the two which occurs entirely within the preserve boundaries. Both ponds were intensively inventoried, but sampling efforts in the present study were focused on the northern pond, which is approximately .69 ha (1.7 acres) in area. A total of 41 taxa were found in the sand pond with 17 of these occurring within sampling plots (Table 2). Sampling was conducted in September at a time when water levels were very low and the majority of the sand pond was an exposed mudflat. Dominant taxa at the time of surveys (those with IV 200% > 20.0%) were, in descending rank order, *Glyceria septentrionalis* Hitchc. (floating manna grass) (IV 32.5%), *Persicaria coccinea* (Muhl.) Greene (scarlet smartweed) (IV 26.2%), *Bidens cernua* L. (nodding bur marigold) (IV 23.8%) and *Sparganium androcladum* (Engelm.) Morong (bur-reed) (IV 20.4%) (Table 2). Other important taxa included *Echinochloa muricata* (Michx.) Fern. (wild millet) (IV 17.3%), *Pontederia cordata* L. (pickerelweed) (IV 15.2%), *Phalaris arundinacea* L. (reed canary grass) (IV 11.8%), and *Sagittaria brevirostra* Mack. & Bush (short-beaked arrowhead) (IV 11.6%) (Table 2). Had sampling occurred earlier in the growing season when water levels are typically much higher, cer-

tain taxa would have undoubtedly had higher importance values, including *Nuphar advena* (Aiton) W. T. Aiton (yellow pond lily), *Nymphaea tuberosa* Paine (white water lily), and *Ranunculus flabellaris* Raf. (yellow-flowered water buttercup). Additionally, species such as *Callitriche heterophylla* Pursh (large water starwort), and *Lemna minor* L. (common duckweed), which were not present at the time of sampling, would likely have occurred within sampling plots. Several taxa that occurred in sampling plots which would have been absent during high water levels included *Bidens cernua, B. coronata* (L.) Britt. (tall swamp marigold), *B. frondosa* L. (common tickseed), *Echinochloa muricata*, and *Erechtites hieracifolia* (L.) Raf. (fireweed). Only one exotic taxon, *Persicaria hydropiper* (L.) Opiz (water pepper) occurred in the sand pond. The native FQI for this area was 24.3 (24.1 with adventive taxa) and the native $\overline{C}$-value was 3.8 (unchanged with adventives). Although the FQI for this area was somewhat low, due in part to the low number of species within this community type, the higher $\overline{C}$-value of 3.8 is indicative of a noteworthy remnant community that still has a relatively high degree of natural integrity.

Marsh/Wet Sand Prairie Pond Margins

Bordering all sides of both sand ponds were transitional areas where sand pond communities gradually graded into other adjacent community types (Area 5; Figure 1). These areas were slightly more elevated and drier than the ponds, and characterized by zones, often very narrow, of vegetation noticeably different from the vegetation of the community types occurring on either side. These areas were difficult to assign to any one community type, but would be best characterized as a combination of marsh/wet sand prairie. Virtually all areas of this community type had moderate to heavy infestations of *Phalaris arundinacea*. The most diverse area of this type was located on the northern and northeastern margins of the north sand pond between areas 2A and 6 (Figure 1), and occurring here were several taxa with more limited distributions within the preserve, which included *Agrostis gigantea* Roth. (red top), *Boehmeria cylindrica* (L.) Sw. (false nettle), *Carex tribuloides* Wahl. (oval sedge), *Cuscuta polygonorum* Engelm. (knotweed dodder), *Eupatorium perfoliatum* L. (common boneset), *Galium tinctorium* L. (stiff bedstraw), *Leersia oryzoides* (L.) Sw. (rice cut grass), *Spartina pectinata* Link (cord grass), and *Verbena hastata* L. (blue vervain). Sampling results (Table 3) from the zone between areas 2A and 4 (Figure 1) are representative of other marsh/wet sand prairie areas in the preserve and dominant taxa included *Phalaris arundinacea* (IV 43.2%), *Persicaria coccinea* (IV 41.2%), *Bidens cernua* (IV 30.3%), and *Calamagrostis canadensis* (Michx.) P. Beauv. (blue joint grass) (IV 29.8%). Other taxa occurring in this area as well as in this community type throughout the preserve included *Bidens connata* Muhl. (purple-stemmed tickseed), *B. coronata, Bolboschoenus fluviatilis* (Torr.) Sojak, (river bulrush), *Eleocharis palustris* (L.) Roem. & Schult. (great spike rush), *Eupatorium serotinum* Michx. (late boneset), *Persicaria punctata* (Ell.) Small (smartweed), *Salix nigra* Marsh. (black willow), and *Scirpus cyperinus* (L.) Kunth (wool grass).

Wet-mesic Sand Prairie

Located on the southeast corner of BPNP (Area 4; Figure 1) is a moderately degraded wet-mesic sand prairie approximately .74 ha (1.8 acres) in area. A total of 53 taxa were found in this portion of the preserve and 21 of these occurred in the sampling plots (Table 4). Woody stem encroachment is advancing on the eastern and southeastern boundaries of this wet-mesic sand prairie, with the shrub *Rubus pensylvanicus* Poir. (yankee black-

berry) invading most heavily. Other woody species encroaching along these boundaries included *Cornus obliqua* Raf. (blue-fruited dogwood), *Rubus allegheniensis*, *Salix discolor* Muhl. (pussy willow), and *S. nigra* Marsh (black willow). Diversity in these areas was very low and historical aerial photographs ranging from 1940 to 1973 reveal that these areas previously, were completely open. Similarly, along the northern boundary were several large individuals of *Acer saccharinum* L. (silver maple) and *Quercus palustris* Muench (pin oak) which are absent on 1940 aerial photographs. These highly shaded areas were also lacking in diversity and bare ground/leaf litter was abundant.

All areas of the wet-mesic sand prairie not experiencing advanced woody stem encroachment were dominated by *Calamagrostis canadensis* (Table 4), and these areas were somewhat lacking in diversity. Other important taxa in the open areas included *Persicaria coccinea* (IV 39.9%), *Erechtites hieracifolia* (IV 31.6%), *Bidens coronata* (IV 14.2%), and *Eupatorium serotinum* (IV 9.1%). *Phalaris arundinacea*, although having a lower importance value along the sampling transect, was abundant in scattered patches with the most heavily infested areas usually occurring at the boundaries between areas 4 and 5 (Figure 1).

Several taxa within the preserve were only found in the wet-mesic sand prairie community, and infrequently to occasionally encountered species occurring here included: *Acalypha gracilens* Gray (slender three-leaved mercury), *Asclepias incarnata* L. (swamp milkweed), *Carex pellita* Willd. (wooly sedge), *C. scoparia* Schk. (oval sedge), *Epilobium ciliatum* Raf. (willow herb), *Helianthus mollis* Lam. (downy sunflower), *Hypericum mutilum* L. (dwarf St. John's-wort), *Iris shrevei* Small (blue iris), *Ludwigia alternifolia* L. (seedbox), *Lycopus uniflorus* Michx. (northern bugle weed), *Panicum virgatum* L. (switch grass), *Persicaria opelousana* (Riddell) Small (scaly smartweed), *Rhexia virginica* L. (meadow beauty), *Spiraea alba* Du Roi (meadowsweet), *Stachys pilosa* Nutt. var. *homotricha* (Fern.) Mohlenbr. (woundwort), *Vernonia missurica* Raf. (Missouri ironweed), and *Viola lanceolata* L. (lance-leaved violet). The native FQI for this area was 27.2 (26.7 with adventive taxa) and the $\overline{C}$ -value was 3.7 (3.6 with adventive taxa). As with the previously discussed sand pond habitat, despite having a lower FQI value, the higher $\overline{C}$ -value of 3.7 for this community is indicative of an area with noteworthy remnant quality.

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LITERATURE CITED

Anderson, R.C. 1970. Prairies in the prairie state. Transactions of the Illinois State Academy of Science. 63:214-221.

Bailey, A.W. and C.E. Poulton. 1968. Plant communities and environmental relationships in a portion of the Tillamook burn, northwestern Oregon. Ecology 49:1-13.

Christensen, N.L., A.N. Bartuska, J.H. Brown, S. Carpenter, C. D'Antonio, R. Francis, J.F. Franklin, J.A. MacMahon, R.F. Noss, D.J. Parsons, C.H. Peterson, M.G. Turner, and R.G. Woodmansee. 1996. The report of the Ecological Society of America Committee on the Scientific Basis for Ecosystem Management. Ecol. Appl. 6:665-691.

Cowles, H.C. 1899. The ecological relations of the vegetation on the sand dunes of Lake Michigan. Botanical Gazette 27(2, 3, 4, + 5):95-117, 167-202, 281-308, 361-391.

Daubenmire, R. 1959. A canopy coverage method of vegetation analysis. Northwest Science 33:43-64.

Fehrenbacher, J.B., J.D. Alexander, I.J. Jansen, R.G. Darmody, R.A. Pope, M.A. Flock, E. E. Voss, J. W. Scott, W. F. Andrews, and L. J. Bushue. 1984. Soils of Illinois. University of Illinois at Urbana-Champaign College of Agriculture and Agricultural Experiment Station in Cooperation with the Soil Conservation Service, U. S. Department of Agriculture, Bulletin 778. 85 pp. + map.

Fell, E.W. 1957. Plants of a Northern Illinois Sand Deposit. American Midland Naturalist 58(2):441-451.

Frankie, W.T., M.M. Killey, and R.J. Russell. 1996. Guide to the Geology of the Hoopeston Area, Vermilion and Iroquois Counties, Illinois. Illinos State Geological Survey, Field Trip Guidebook 1996B, 54 pp.

Follmer, L. Discussion on the geologic history and quaternary deposits of Illinois, with Leon Follmer of the Illinois State Geological Survey, March, 2006. Champaign, Illinois.

Gates, F.C. 1910. Relic dunes, a life history. Transactions of the Illinois State Academy Science 3:110-116.

Gates, F.C. 1912. The vegetation of the beach area in northeastern Illinois and southeastern Wisconsin. Illinois State Laboratory of Natural History Bulletin 9:255-372.

Gleason, H.A. 1909. The Vegetational History of a River Dune. Transactions of the Illinois State Academy of Science 2:19-26.

Gleason, H.A. 1910. The Vegetation of the Inland Sand Deposits of Illinois. Illinois State Laboratory of Natural History Bulletin 9:23-174.

Hansel, A.K., and W.H. Johnson. 1996. Wedron and Mason Groups: Lithostratigraphic reclassification of deposits of the Wisconsin Episode, Lake Michigan Lobe Area. Illinois State Geological Survey Bulletin 104. 116 pp.

Hart, C.A. and H.A. Gleason. 1907. On the Biology of the Sand Areas of Illinois. Illinois State Laboratory of Natural History Bulletin 7:137-272.

Higley, W.K. and C.S. Raddin. 1891. Flora of Cook County, Illinois and a part of Lake County, Indiana. Bulletin of the Chicago Academy of Sciences 2(1):1-168.

Iverson, L.R. 1988. Land-use changes in Illinois USA: the influence of landscape attributes on current and historic landuse. Landscape Ecology 2:45-61.

Kiefer, L.M. 1982. Soil survey of Iroquois County, Illinois. U. S. Department of Agriculture, Soil Conservation Service and Illinois Agricultural Experiment Station, University of Illinois at Urbana-Champaign, Soil Report No. 115.

Killey, M.M. 1998. Illinois' Ice Age Legacy. Illinois State Geological Survey; Geoscience Education Series 14. 66 pp.

Lineback, J.A. 1979. Quaternary deposits of Illinois. Illinois State Geological Survey Map.

McDonald, F.E. 1900. A sand dune flora of Central Illinois. Plant World 3(7):101-103.

Midwestern Regional Climate Center 2006. http://mcc.sws.uiuc.edu

Mohlenbrock, R.H. 2002. Vascular Flora of Illinois. Southern Illinois University Press, Carbondale, IL. 490 pp.

Szafoni, D.L., D.M. Greer, and L. Cordle. 2002. Land Cover of Illinois in the Early 1800s. INHS GIS poster and database.

Schwegman, J.E. 1973. Comprehensive plan for the Illinois nature preserves system. Part 2. The natural divisions of Illinois. Illinois Nature Preserves Commission, Rockford, Illinois. 32 pp. + map.

Swink, F. and G. Wilhelm. 1994. Plants of the Chicago region, 4th ed. The MortonArboretum, Lisle, Illinois, and the Indiana Academy of Science, Indianapolis. 921 pp.

Taft, J.B., G.S. Wilhlem, D.M. Ladd, and L.A. Masters. 1997. Floristic quality assessment for vegetation in Illinois. A method for assessing vegetation integrity. Erigenia 15:3-95.

Vestal, A.G. 1931. A preliminary vegetation map of Illinois. Transactions of the Illinois State Academy of Science 23:204-217.

White, J. 1978. Illinois Natural Areas Inventory. Technical Report. Volume 1. Survey methods and results. Natural Areas Inventory, Urbana. xix + 426 pp.

White, J. and M.H. Madany. 1978. Classification of natural communities in Illinois. Pages 310-405 in Illinois Natural Areas Inventory. Technical Report (J. White, ed.). Illinois Natural Areas Inventory, Urbana.

Wiggers, R. 1997. Geology underfoot in Illinois. Mountain Press Publishing. Missoula Montana. 303 pp.

Willman, H.B. and J.C. Frye. 1970. Pleistocene stratigraphy of Illinois. Illinois State Geological Survey Bulletin 94:1-204.

APPENDIX I.

Vascular plant taxa encountered at Bonnie's Prairie Nature Preserve. Pteridophytes are listed first, followed by angiosperms. Angiosperms are further divided into monocots and dicots. Familes, genera and species are arranged alphabetically within groups. Adventive taxa are indicated by an asterisk (*). Following the binomial and authority, the community type(s) in which each taxa occurred, is indicated. Community designations correspond to areas shown in Figure 1. Designations are: [1=dry sand prairie (A-higher quality, B-highly degraded); 2=sand pond (A-deeper with higher diversity, B-shallower with lower diversity); 3=dry-mesic sand prairie (highly degraded); 4=wet-mesic sand prairie; 5=marsh/wet sand prairie; 6=scrubland (highly degraded successional); 7=dry sand savanna (A-inclusion in higher quality sand prairie, B-inclusion in highly degraded sand prairie); and 8=margins of preserve (E-east margin along railroad, N-north margin along road)]. Nomenclature follows Mohlenbrock (2002). Collecting numbers are those of M. J. C. Murphy (M). All specimens are deposited in the Illinois Natural History Survey Herbarium (ILLS), Champaign, IL.

FERNS AND FERN-ALLIES
Aspleniaceae
Asplenium platyneuron (L.) Oakes: 7; M207
Equisetaceae
Equisetum arvense L.: 3; M636
Equisetum hyemale L. ssp. *affine* Calder & Taylor: 3; M635
Onocleaceae
Onoclea sensibilis L.: 6; M129
Thelypteridaceae
Thelypteris palustris Schott: 6; M607

ANGIOSPERMS
MONOCOTS
Alismataceae
Alisma subcordatum Raf.: 2A, 2B; M571
Sagittaria brevirostra Mack. & Bush.: 2A, 2B; M567
Sagittaria graminea Michx.: 2A, 2B; M221
Commelinaceae
**Commelina communis* L.: 6; M543
Tradescantia ohiensis Raf.: 1A; M107
Cyperaceae
Bolboschoenus fluviatilis (Torr.) Sojak: 2B, 5; M284
Carex brevior (Dewey) Lunell: 1A, 8E; M109
Carex emmonsii Dewey: 6; M134
Carex muhlenbergii Schk. var. *muhlenbergii*: 1A; M198
Carex pellita Willd.: 4; M281
Carex scoparia Schk.: 4; M837
Carex swanii (Fern.) Mack.: 4, 6; M132
Carex tribuloides Wahl.: 5; M559
Carex vulpinoidea Michx.: 4, 5; M277
Cyperus erythrorhizos Muhl.: 2B; M816
Cyperus lupulinus (Spreng.) Marcks var. *lupulinus*: 1A; M521
Cyperus schweinitzii Torr.: 3; M825
Cyperus strigosus L.: 2A, 4; M811
Eleocharis ovata (Roth) Roem. & Schult. var. *obtusa* (Willd.) Kukenth: 2A, 2B; M806
Eleocharis palustris (L.) Roem. & Schultes: 2A, 2B, 4, 5; M570
Schoenoplectus heterochaetus (Chase) Sojak: 2A, 2B, 5; M149
Schoenoplectus tabernaemontani (C. C. Gmel.) Palla: 2A; M572
Scirpus cyperinus (L.) Kunth: 5; M566
Iridaceae
Iris shrevei Small: 4; M805
Juncaceae
Juncus acuminatus Michx.: 3; M631
Juncus biflorus Ell. f. *biflorus*: 3; M621
Juncus brachycarpus Englem.: 3; M627
Juncus greenei Oakes & Tuckerm.: 3; M622
Juncus interior Wieg.: 1A; M194
Juncus tenuis Willd.: 1A, 4; M591
Luzula bulbosa (A. W. Wood) Smyth.: 6; M133
Lemnaceae
Lemna minor L.: 2A, 2B; M22
Liliaceae
**Asparagus officinalis* L.: 1A, 7A; M119
Polygonatum commutatum (Schult.) A. Dietr.: 1A, 7A; M120
Smilacina stellata (L.) Desf.: 1A, 7A; M117

Poaceae
Agrostis hyemalis (Walt.) BSP.: 1A, 8E; M189
Agrostis gigantea Roth.: 4, 5; M558
Agrostis perennans (Walter) Tuck.: 6; M608
Andropogon gerardii Vitman: 1A; M761
Andropogon virginicus L.: 3; M813
Aristida purpurascens Poir.: 1A; M756
**Bromus commutatus* Schrad.: 1A, 8E; M187
**Bromus inermis* Leyss: 1A, 8E; M121
**Bromus tectorum* L.: 1A, 8E; M103
Calamagrostis canadensis (Michx.) P. Beauv.: 2A, 2B, 4, 5; M214
Cenchrus longispinus (Hack.) Fern.: 3; M826
Dichanthelium acuminatum (Sw.) Gould & Clark: 1A; M193
Dichanthelium oligosanthes (Schult.) Gould var. *scribnerianum* (Nash) Gould: 1A; M126
Dichanthelium villosissimum (Nash) Freckm.: 1A; M201
**Digitaria sanguinalis* (L.) Scop.: 2B; M818
Echinochloa muricata (Michx.) Fern.: 2A, 2B; M774
Elymus canadensis L.: 1A; M548
Eragrostis pectinacea (Michx.) Nees: 1A, 8E; M762
Eragrostis spectabilis (Pursh) Steud.: 1A, 1B; M611
Glyceria septentrionalis Hitchc.: 2A, 2B, 5; M211
Heterostipa spartea (Trin. & Rupr.) Barkworth: 1A; M122
Koeleria macrantha (Ledeb.) Spreng.: 1A, 8E; M96
Leersia oryzoides (L.) Swartz: 2A, 5; M786
Leptoloma cognatum (Schult.) Chase: 1A, 8E; M527
Panicum dichotomiflorum Michx.: 2B; M817
Panicum rigidulum Bosc var. *rigidulum*: 6; M609
Panicum virgatum L.: 4; M579
*Paspalum setaceum*Michx. var. *ciliatifolium* (Michx.) Vasey: 1A; M824
**Poa compressa* L.: 1A, 1B, 3, 4, 7A & B, 8E, 8N; M530
**Poa pratensis* L.: 1A, 1B, 3, 6, 7, 8E, 8N; M114
Phalaris arundinacea L.: 2A, 2B, 4, 5, 6, 8E; M136
Schizachyrium scoparium (Michx.) Nash: 1A, 1B, 3; M829
Sorghastrum nutans (L.) Nash: 1A; M755
Spartina pectinata Link: 5; M557
Sporobolus cryptandrus (Torr.) Gray: 1A; M760
Sporobolus heterolepis (Gray) Gray: 1A; M759
Tridens flavus (L.) Hitch.: 1A, 1B, 8E, 8N; M765
Triplasis purpurea (Walt.) Chapm.: 1B, 3; M624
Pontederiaceae
Pontederia cordata L.: 2A, 2B; M569
Sparganiaceae
Sparganium androcladum (Engelm.) Morong: 2A, 2B; M568
Sparganium eurycarpum Englem.: 2A, 2B, 5; M601
Typhaceae
Typha latifolia L.: 2A, 5; M273

DICOTS
Aceraceae
Acer saccharinum L.: 4, 5, 6; M137
Anacardiaceae
Rhus glabra L.: 1A, 7A; M202
Rhus hirta L.: 6; M612
Toxicodendron radicans (L.) Kuntze: 1A, 1B, 3, 4, 6, 7A, 7B; M603
Apiaceae
**Daucus carota* L.: 1A, 1B, 3, 8E, 8N; M541
Sanicula canadensis L. var. *canadensis*: 8N; M639
Sium suave Walt.: 2A, 2B, 5; M564
Asclepiadaceae
Asclepias amplexicaulis Small: 1A; M209
Asclepias incarnata L.: 4; M1853
Asclepias syriaca L.: 1A, 1B, 3, 8E, 8N; M828
Asclepias verticillata L.: 1A, 1B, 3; M768
Asteraceae
**Achillea millefolium* L.: 1A, 1B, 3, 8E, 8N; M124
Ambrosia artemisiifolia L.: 1A, 1B, 4, 3, 8E, 8N; M526
Antennaria neglecta Greene: 1A, 1B; M268
Antennaria plantaginifolia (L.) Hook.: 1A, 1B; M267
Aster pilosus Willd.: 1A, 1B, 3, 8E, 8N; M753
Bidens cernua L.: 2A, 2B, 5; M773
Bidens connata Muhl.: 2A, 2B, 5; M777
Bidens coronata (L.) Britt.: 2A, 2B, 4, 5; M778
Bidens frondosa L.: 2A, 2B, 4, 5; M771
Boltonia asteroides (L.) L' Her. Var. *recognita* (Fern. & Grisc.) Cronq.: 2B/5; M1853

Cirsium discolor (Muhl.) Spreng.: 1A; M763
Conyza canadensis (L.) Cronq.: 3, 8E, 8N; M525
Erechtites hieracifolia (L.) Raf.: 2A, 2B, 4, 5; M575
Erigeron strigosus Muhl.: 1A, 1B; M208
Eupatorium perfoliatum L.: 4, 5; M604
Eupatorium serotinum Michx.: 4, 5, 8E; M556
Euthamia gymnospermoides Greene: 3, 4; M781
Helianthus mollis Lam.: 4; M583
Helianthus occidentalis Riddell: 1A; M757
Hieracium gronovii L.: 3; M630
Lactuca canadensis L.: 1A, 7A; M545
Liatris aspera Michx.: 1A; M787
Pseudognaphalium obtusifolium (L.) Hilliard & Burtt.: 3; M820
Solidago altissima L.: 1A; 3, 4, 8E, 8N; M764
Vernonia missurica Raf.: 4; M581

Betulaceae
Bignoniaceae
Boraginaceae
Brassicaceae
Caesalpiniaceae
Callitrichaceae
Cannabinaceae
Caprifoliaceae
Caryophyllaceae
Chenopodiaceae
Cistaceae
Cornaceae
Corylaceae
Cuscutaceae
Elaeagnaceae
Euphorbiaceae
Fabaceae
Fagaceae
Geraniaceae
Grossulariaceae
Hypericaceae

**Betula populifolia* Marsh.: 6; M135
**Catalpa speciosa* Warder: 1A/8E; M195
Hackelia virginiana (L.) I. M. Johnst.: 6; M628
Lithospermum croceum Fern.: 1A, 8E; M110
Myosotis verna Nutt.: 6; M131
Cardimine parviflora L. var. *arenicola* (Britt.) O.E. Schultz: 6; M153
Lepidium virginicum L.: 1A, 1B, 8E; M101
Rorippa palustris (L.) Besser: 2A; Observed
Chamaecrista fasciculata (Michx.) Greene: 1A, 8E, 8N; M524
Callitriche heterophylla Pursh: 2A; M216
**Cannabis sativa* L.: 6; M573
**Lonicera maackii* (Rupr.) Maxim.: 1A, 6, 7A, 8N; M640
Sambucus canadensis L.: 6; M552
**Silene pratensis* (Spreng.) Godron & Gren.: 1A, 8E, 8N; M522
Chenopodium desiccatum A. Nels.: 1A; M767
Helianthemum canadense (L.) Michx.: 1A; M204
Lechea mucronata Raf.: 1A; M766
Lechea pulchella Raf.: 3; M619
Cornus obliqua Raf.: 4, 5, 6; M553
Corylus americana Walt.: 6; M606
Cuscuta polygonorum Engelm.: 2A, 2B, 4, 5; M562
**Elaeagnus umbellata* Thunb.: 6; M614
Acalypha gracilens Gray: 4; M588
Acalypha rhomboidea Raf.: 3, 4, 6; M814
Chamaesyce maculata (L.) Small: 3; M827
Chamaesyce nutans (Lag.) Small: 8N; M830
Euphorbia corollata L.: 1A, 8E, 8N; M265
Poinsettia dentata (Michx.) Kl. & Garcke: 1A; M520
Amorpha canescens Pursh: 1A; M266
Apios americana Medic.: 5, 8N; M537
Crotolaria sagittalis L.: 3; M618
Dalea purpurea Vent.: 1A; Observed
Desmodium illinoense Gray: 1A, 3; M752
Desmodium sessilifolium (Torr.) Torr. & Gray: 3; M822
Lespedeza capitata Michx.: 1A, 3; M531
Lespedeza hirta (L.) Hornem.: 1A; M542
**Melilotus alba* Medic.: 1A, 1B, 3, 8E, 8N; M190
Strophostyles helvula (L.) Ell.: 1A; M528
Strophostyles leiosperma (Torr. & Gray) Piper: 1A; M532
Tephrosia virginiana (L.) Pers.: 1A, 8E; M185
Quercus palustris Muench: 4, 5, 6; M544
Quercus velutina Lam.: 1A, 1B, 3, 6, 7A, 7B; M533
Geranium carolinianum L.: 1A, 8N; M108
Ribes missouriense Nutt.: 6; M551
Hypericum mutilum L.: 4; M576

Lamiaceae
Lycopus americanus Muhl.: 4, 5; M582
Lycopus uniflorus Michx.: 4; M593
Monarda punctata L.: 1A, 1B; M539
Prunella vulgaris L. var. *elongata* Benth.: 3, 6, 8N; M629
Scutellaria lateriflora L.: 6; M597
Stachys pilosa Nutt. var. *homotricha* (Fern.) Mohlenbr.: 4; M274
Lauraceae
Sassafras albidum (Nutt.) Nees: 1A, 6, 7A, 7B, 8E, 8N; M16
Linaceae
Linum medium (Planch.) Britt.: 3; M616
Lythraceae
Rotala ramosior (L.) Koehne: 2A; M808
Melastomaceae
Rhexia virginica L.: 4; M594
Molluginaceae
**Mollugo verticillata* L.: 3; M633
Moraceae
**Morus alba* L.: 1A, 6, 7A, 8N; M799
Nyctaginaceae
**Mirabilis nyctaginea* (Michx.) MacM.: 1A, 3; M206
Nymphaeaceae
Nuphar advena (Ait.) Ait. f.: 2A, 2B; M148
Nymphaea tuberosa Paine: 2A, 2B; M147
Oleaceae
Fraxinus lanceolata Borkh.: 6; M838
Fraxinus pennsylvanica Marsh.: 3, 6; M610
Onagraceae
Circaea lutetiana Aschers. & Magnus: 6; M602
Epilobium ciliatum Raf.: 4; M590
Ludwigia alternifolia L.: 4; M589
Ludwigia palustris (L.) Ell.: 2B; M596
Ludwigia polycarpa Short & Peter.: 2A; M807
Oenothera biennis L.: 3, 4, 8E, 8N; M577
Oenothera clelandii W. Dietr., Raven & W. L. Wagner: 1A; M769
Oxalidaceae
Oxalis stricta L.: 1A, 1B, 3, 6, 8E, 8N; M111
Phytolaccaceae
Phytolacca americana L.: 6, 8E; M796
Plantaginaceae
**Plantago lanceolata* L.: 1A, 6, 8E, 8N; M270
**Plantago patagonica* Jacq.: 1A; M203
Plantago virginica L.: 1A; M100
Polemoniaceae
Phlox bifida Beck.: 1A, 8N, 8E; M15
Polygalaceae
Polygala sanguinea L.: 3; M615
Polygonaceae
**Fallopia convolvulus* (L.) A. Love: 8N; M538
Persicaria coccinea (Muhl.) Greene: 2A, 2B, 4, 5; M563
**Persicaria hydropiper* (L.) Opiz: 2A; M809
Persicaria hydropiperoides (Michx.) Small: 2A, 2B, 5; M775
Persicaria lapathifolia (L.) S. F. Gray: 2A; M776
Persicaria opelousana (Riddell) Small: 4; M586
Persicaria pensylvanica (L.) Small: 2A, 2B, 5; M592
Persicaria punctata (Ell.) Small: 2A, 2B, 4, 5; M561
**Persicaria vulgaris* Webb & Moq.: 3; M634
**Rumex acetosella* L.: 1A, 1B, 3, 8E, 8N; M116
Rumex verticillatus L.: 2A, 5; M218
Portulacaceae
Claytonia virginica L.: 1A, 6; M14
Ranunculaceae
Anemone cylindrica Gray: 1A; M199
Ranunculus flabellaris Raf.: 2A, 2B; M146
Rosaceae
Agrimonia parviflora Sol.: 3, 4; M584
Crataegus crus-galli L.: 1A; M754
Fragaria virginiana Duchesne: 1A, 1B, 3, 6, 7A; M115
Geum canadense Jacq.: 6; M536
Potentilla simplex Michx.: 1A, 1B, 6; M112
**Potentilla recta* L.: 1A; M200
Prunus serotina Ehrh.: 1A, 6, 7A, 7B; M130
Rosa carolina L.: 1A; M196
**Rosa multiflora* Thunb.: 6, 8E; M141
Rosa X *rudiuscula* Greene: 4; M585
Rosa setigera Michx.: 6; M269
Rubus allegheniensis Porter: 1A, 4, 6; M118
Rubus flagellaris Willd.: 1A, 6; M264
**Rubus laciniatus* Willd.: 6; M605
Rubus occidentalis L.: 6; M127
Rubus pensilvanicus Poir.: 4; M280
Spiraea alba Du Roi: 4; M287
Rubiaceae
Diodia teres Walt.: 1A, 1B, 3; M758
Galium aparine L.: 1A, 6, 8; M102
Galium circaezans Michx. var. *hypomalacum* Fern.: 6, 7A; M535
Galium tinctorium L.: 2A, 4, 5; M565
Salicaceae
Populus deltoides Marsh: 3, 6, 8E; M801
Salix amygdaloides Anders.: 5, 6; M210
Salix discolor Muhl.: 3, 4, 6; M823
Salix interior Rowlee: 6; M599

Salix nigra Marsh.: 2A, 4, 5, 6; M213
Scrophulariaceae
Agalinis besseyana Britt.: 3; M632
Gratiola virginiana L.: 2A: M1857
Lindernia anagallidea (Michx.) Pennell: 2A; M810
Scrophularia lanceolata Pursh: 1A, 7A; M123
Solanaceae
Physalis virginiana Mill.: 1A; M197
Solanum carolinense L.: 4; M275
**Solanum dulcamara* L.: 8E; M139
Solanum ptychanthum Dunal.: 8E; M800
Urticaceae
Boehmeria cylindrica (L.) Sw.: 5, 6; M555
Verbenaceae
Verbena hastata L.: 4, 5; M554
Violaceae
Viola lanceolata L.: 4; M151
Viola sagittata Aiton: 6; M271
Vitaceae
Parthenocissus quinquefolia (L.) Planch.: 1A, 1B, 3, 4, 6, 7A, 7B, 8E, 8N; M637
Vitis riparia Michx.: 1A, 3, 4, 5, 6, 7A, 8E, 8N; M125

Figure 1. Natural communities of Bonnie's Prairie Nature Preserve, Iroquois County, Illinois. 1=dry sand prairie (A-higher quality, B-highly degraded); 2=temporary sand pond (A-deeper with higher diversity, B-shallower with lower diversity); 3=dry-mesic sand prairie (highly degraded); 4=wet-mesic sand prairie; 5=marsh/web sand prairie; 6=scrubland (highly degraded successional); 7=dry sand savanna (A-eastern inclusion in higher quality sand prairie, B-western inclusion in more degraded sand prairie); 8=margins of preserve (N-north margin along road, E-eastern margin along railroad tracks). Paired arrows show locations of sampling transects within community types.

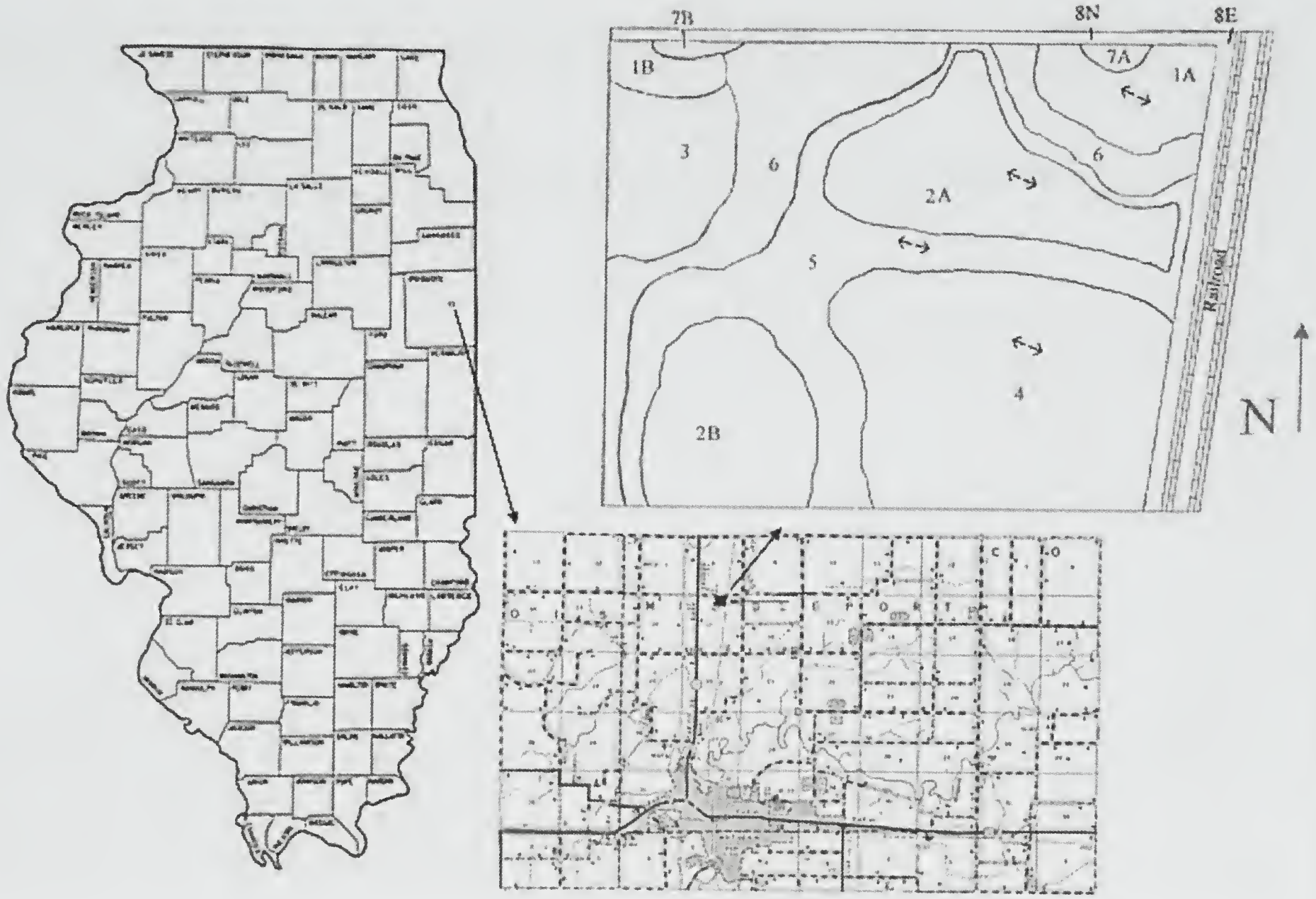

Table 1. Frequency, mean cover, relative frequency, relative cover, and importance values (IV 200) of ground flora species occurring in dry sand prairie sampling plots at Bonnie's Prairie Nature Preserve, Iroquois Co., Illinois. Species are listed in descending rank order by importance value. Importance values are the sum of the relative frequency and relative cover (* = adventive species).

Species	Freq. %	Mean Cover	Rel.Freq. %	Rel.Cover %	IV 200 (%)
Schizachyrium scoparium	100	17.91	9.28	27.29	36.6
**Achillea millefolium*	100	11.40	9.28	17.37	26.6
Leptoloma cognatum	82	8.12	7.61	12.37	20.0
**Poa pratensis*	96	4.63	8.91	7.05	16.0
Rubus flagellaris	60	5.01	5.57	7.63	13.2
Phlox bifida	94	2.74	8.72	4.17	12.9
**Rumex acetosella*	56	3.90	5.19	5.94	11.1
Ambrosia artemisiifolia	64	1.64	5.94	2.50	8.4
Cyperus lupulinus var. lupulinus	70	1.10	6.49	1.68	8.2
Aster pilosus	28	1.36	2.60	2.07	4.7
Lithospermum croceum	24	1.29	2.23	1.97	4.2
**Bromis inermis*	20	1.51	1.86	2.30	4.2
Dichanthelium oligosanthes var. scribnerianum	28	0.92	2.60	1.40	4.0
Helianthemum canadense	26	0.53	2.41	0.81	3.2
Carex muhlenbergii var. muhlenbergii	24	0.27	2.23	0.41	2.6
Euphorbia corollata	22	0.31	2.04	0.47	2.5
Paspalum setaceum var. ciliatifolium	18	0.53	1.67	0.81	2.5
Dichanthelium villosissimum	16	0.33	1.48	0.50	2.0
Sporobolus cryptandrus	16	0.13	1.48	0.20	1.7
Physalis virginiana	14	0.17	1.30	0.26	1.6
**Potentilla recta*	14	0.17	1.30	0.26	1.6
Lespedeza capitata	12	0.11	1.11	0.17	1.3
Potentilla simplex	10	0.20	0.93	0.30	1.2
Oxalis stricta	12	0.06	1.11	0.09	1.2
Tradescantia ohiensis	10	0.05	0.93	0.08	1.0
Sorghastrum nutans	8	0.09	0.74	0.14	0.9
Sassafras albidum	6	0.18	0.56	0.27	0.8
Aristida purpurascens	8	0.04	0.74	0.06	0.8
Amorpha canescens	2	0.30	0.19	0.46	0.6
Antennaria plantaginifolia	2	0.30	0.19	0.46	0.6
Diodia teres	6	0.03	0.56	0.05	0.6
Strophostyles leiosperma	6	0.03	0.56	0.05	0.6
Conyza canadensis	4	0.02	0.37	0.03	0.4
Dalea purpurea	4	0.02	0.37	0.03	0.4
Cirsium discolor	2	0.06	0.19	0.09	0.3
Desmodium illinoense	2	0.06	0.19	0.09	0.3
Rosa carolina	2	0.06	0.19	0.09	0.3
Antennaria neglecta	2	0.01	0.19	0.02	0.2
Asclepias verticillata	2	0.01	0.19	0.02	0.2
Lechea mucronata	2	0.01	0.19	0.02	0.2
Oenothera clelandii	2	0.01	0.19	0.02	0.2
Poinsettia dentata	2	0.01	0.19	0.02	0.2
Totals		65.63	100.00	100.00	200.0
Bare ground and litter		31.42			

Table 2. Frequency, mean cover, relative frequency, relative cover, and importance values (IV 200) of ground flora species occurring in sand pond (northern) sampling plots at Bonnie's Prairie Nature Preserve, Iroquois Co., Illinois. Species are listed in descending rank order by importance value. Importance values are the sum of the relative frequency and relative cover.

Species	Freq. %	Mean Cover	Rel.Freq. %	Rel.Cover %	IV 200 (%)
Glyceria septentrionalis	88	13.34	12.15	20.38	32.5
Persicaria coccinea	72	10.66	9.94	16.28	26.2
Bidens cernua	96	6.90	13.26	10.54	23.8
Sparganium androcladum	48	9.04	6.63	13.81	20.4
Echinochloa muricata	68	5.20	9.39	7.94	17.3
Pontederia cordata	48	5.60	6.63	8.55	15.2
Phalaris arundinacea	44	3.72	6.08	5.68	11.8
Sagittaria brevirostra	52	2.90	7.18	4.43	11.6
Persicaria hydropiperoides	24	3.06	3.31	4.67	8.0
Ranunculus flabellaris	48	0.64	6.63	0.98	7.6
Nymphaea tuberosa	32	1.92	4.42	2.93	7.4
Acer saccharinum	36	0.18	4.97	0.27	5.2
Nuphar advena	20	1.08	2.76	1.65	4.4
Erechtites hieracifolia	24	0.42	3.31	0.64	4.0
Leersia oryzoides	12	0.74	1.66	1.13	2.8
Bidens frondosa	8	0.04	1.10	0.06	1.2
Bidens coronata	4	0.02	0.55	0.03	0.6
Totals		65.46	100.00	100.00	200.0
Bare ground and litter		15.36			

Table 3. Frequency, mean cover, relative frequency, relative cover, and importance values (IV 200) of ground flora species occurring in marsh/wet sand prairie pond margin sampling plots at Bonnie's Prairie Nature Preserve, Iroquois Co., Illinois. Species are listed in descending rank order by importance value. Importance values are the sum of the relative frequency and relative cover.

Species	Freq. %	Mean Cover	Rel.Freq. %	Rel.Cover %	IV 200 (%)
Phalaris arundinacea	76	28.98	12.67	30.55	43.2
Persicaria coccinea	100	23.24	16.67	24.50	41.2
Bidens cernuua	80	16.12	13.33	16.99	30.3
Calamagrostis canadensis	88	14.38	14.67	15.16	29.8
Bidens connata	52	5.62	8.67	5.92	14.6
Eleocharis palustris	68	0.84	11.33	0.89	12.2
Scirpus cyperinus	24	3.54	4.00	3.73	7.7
Persicaria punctata	24	0.22	4.00	0.23	4.2
Eupatorium serotinum	24	0.12	4.00	0.13	4.1
Quercus palustris	20	0.3	3.33	0.32	3.6
Bidens frondosa	12	0.36	2.00	0.38	2.4
Schoenoplectus heterochaetus	12	0.26	2.00	0.27	2.3
Erechtites hieracifolia	8	0.14	1.33	0.15	1.5
Bidens coronata	4	0.6	0.67	0.63	1.3
Leersia oryzoides	4	0.12	0.67	0.13	0.8
Acer saccharinum	4	0.02	0.67	0.02	0.7
Totals		65.46	100.00	100.00	200.0
Bare ground and litter		15.36			

Table 4. Frequency, mean cover, relative frequency, relative cover, and importance values (IV 200) of ground flora species occurring in wet-mesic sand prairie sampling plots at Bonnie's Prairie Nature Preserve, Iroquois Co., Illinois. Species are listed in descending rank order by importance value. Importance values are the sum of the relative frequency and relative cover.

Species	Freq. %	Mean Cover	Rel.Freq. %	Rel.Cover %	IV 200 (%)
Calamagrostis canadensis	100	66.10	21.65	59.86	81.5
Persicaria coccinea	98	20.62	21.21	18.67	39.9
Erechtities hieracifolia	86	14.31	18.61	12.96	31.6
Bidens coronata	46	4.63	9.96	4.19	14.1
Eupatorium serotinum	32	2.44	6.93	2.21	9.1
Persicaria opelousanum	22	0.26	4.76	0.24	5.0
Phalaris arundinacea	12	0.95	2.60	0.86	3.5
Persicaria punctatum	14	0.17	3.03	0.15	3.2
Viola lanceolata	10	0.49	2.16	0.44	2.6
Acalypha rhomboidea	10	0.05	2.16	0.05	2.2
Quercus palustris	8	0.14	1.73	0.13	1.9
Cuscuta polygonorum	4	0.02	0.87	0.02	0.9
Hypericum mutilum	4	0.02	0.87	0.02	0.9
Acer saccharinum	2	0.06	0.43	0.05	0.5
Ambrosia artemisiifolia	2	0.06	0.43	0.05	0.5
Cornus obliqua	2	0.06	0.43	0.05	0.5
Carex swanii	2	0.01	0.43	0.01	0.4
Cyperus strigosus	2	0.01	0.43	0.01	0.4
Dichanthelium acuminatum	2	0.01	0.43	0.01	0.4
Eleocharis palustris	2	0.01	0.43	0.01	0.4
Galium tinctorium	2	0.01	0.43	0.01	0.4
Totals		110.43	100.00	100.00	200.0
Bare ground and litter		13.07			

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Physiologically Based Control of Invasive Asiatic Shrub Honeysuckle

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ABSTRACT

Asiatic shrub honeysuckles (*Lonicera maackii, L.* x *bella* complex) are widespread invaders of Midwestern forests, damaging both plant and animal habitat. Techniques for the elimination of honeysuckle are not well evaluated in the literature. Stem cutting with application of 20% glyphosate to the stubs is widely used, but is not completely successful. It is also fairly painstaking, uses significant quantities of concentrated herbicide, and is purportedly ineffective in spring. Our objective was to develop control techniques which capitalized on the physiological characteristics of understory shrubs, relying on a timed cutting regime and limited or no herbicide application. In one experiment we evaluated whether cutting after leaf expansion followed by foliar spraying or cutting regrowth were effective means of control. In a second experiment we evaluated the effect of cutting height and regrowth spraying on control. Cutting near ground level just after leaf expansion followed by spraying or cutting regrown foliage with dilute (0.9%) glyphosate proved as effective as published results for stem cutting and concentrated glyphosate application. There are significant benefits to adopting early season cutting and midsummer retreatment as a control technique, particularly for private landowners.

INTRODUCTION

Asiatic shrub honeysuckles (*Lonicera maackii* and the *L.* x *bella* hybrid complex = *L. tatarica* x *L. morrowii*) are widespread invaders of forest habitats throughout eastern North America. They often achieve superabundance in forest edges and disturbed stands, excluding native plant species (Woods 1993, Gould and Gorchov 2000, Collier *et al.* 2002) and degrading wildlife habitat (Ingold and Craycraft 1983, White and Stiles 1992, Schmidt and Whelan 1999). Land managers in the Midwest are particularly likely to encounter honeysuckle invasions because the fragmented landscape offers ideal habitat for avian fruit dispersers, and provides plenty of forest edge and disturbed forest (Luken and Goessling 1995).

Honeysuckle extermination is difficult for a number of reasons. The shrub is hard to kill completely (Batcher and Stiles 2000), and resprouts readily (Luken and Mattimiro 1991). Many invaded stands are physically challenging, featuring numerous individuals (e.g., 1800 ha^{-1}, Schulz, unpublished data) of large size (2-5 m in height). Typically, desirable

species are also present, limiting widespread herbicide application or mechanical disturbance. After populations have been exterminated, reestablishment from bird dispersed seed is a continuing concern (Luken and Goessling 1995, White and Stiles 1992).

Based on conversations with resource managers and other professionals, an array of honeysuckle control techniques have been attempted, but few are described, much less evaluated outside the "grey" literature. A few techniques are effective, although each has its drawbacks: In an appropriate situation fire can be used to control honeysuckle, although repeated fires or other secondary treatment are nearly always necessary for satisfactory results (Nyboer 1992). Foliar spraying with 1.5% glyphosate is effective when applied to mature shrubs just after flowering (Nyboer 1992), however there is an obvious practical problem treating large shrubs while minimizing overspray on nontarget species. Honeysuckles can be pulled from soft moist soils, although soil disturbance produces seed beds for weedy species and opportunities for reinfestation (IPAW 2007).

Kline (1981) originated what appears to be the most widely recommended extermination technique: cutting stems at ground level in summer (or later) and painting the stumps with concentrated glyphosate. She achieved an 89.0% mortality rate using a 1:5 glyphosate:water solution on *L.* x *bella* in southern Wisconsin. Reportedly this technique kills honeysuckle even in the winter dormant season (Missouri Conservation Commission 2007), although its efficacy in a broad range of situations is not documented in the wider literature. The Missouri Conservation Commission (2007) comments that glyphosate stump treatments on *L. maackii* are less effective in spring because resources are directed toward new buds as opposed to roots. Reduced effectiveness in spring was not observed by Love *et al.* (2006) working on *L. morrowii* in Pennsylvania.

Although it is part of popular practice, the choice of glyphosate as a *stump treatment* and its use throughout the year has little theoretical support. Kline's rationale for choosing glyphosate pivoted on its benign characteristics during an era when there were few alternatives (Schulz, personal communication). Glyphosate formulated as Roundup®, Kleenup®, and other mixtures has low toxicity to mammals (*Rattus* oral $LD_{50} > 5000$ mg kg^{-1}), and is apparently benign to a wide array of *terrestrial* organisms (Monsanto 2006) (cf. Relyea 2005a,b). It is recommended for foliar application (Roundup® label directions), being readily absorbed by leaves and translocated through both xylem and phloem to meristems. Direct injection into large honeysuckle stems is effective, but this requires specialized apparatus (Hartman and McCarthy 2004). Severed vascular tissue is obviously not ideal for uptake, evidenced by the fact that concentrations for stump treatment are 10 to 20 times stronger than foliar sprays.

Glyphosate functions by inhibiting amino acid synthesis and is most effective in rapidly growing plants (Shaner 2006). Herbicide treatments in summer, fall, and later do not coincide with rapid plant growth. Growth rates of most temperate woody species are greatest in spring (Kozlowski and Pallardy 1997). In the lower Midwest, moisture stress during midsummer caused by higher temperatures and reductions in rainfall (NCDC 2007) potentially reduce physiological activity (Abrams and Mostoller 1995, Kozlowski and Pallardy 1997). Kline (1981) noted vegetative growth resumed during fall in *L.* x *bella* populations near Madison, WI. Based on our observations in southwestern Illinois,

this phenomenon does not generalize to populations of *L. maackii,* and may not apply elsewhere.

From the standpoint of environmental protection, expense, and practicality, additional issues deserve notice. Glyphosate is strongly adsorbed in soil, but persists a longer period (60 d) than many herbicides used in forest management (McNabb 1996). Limiting discharge of glyphosate is desirable because it is not only toxic to all plants but there is evidence that the Roundup® formulation is toxic to amphibian larvae (Relyea 2005a,b). Glyphosate formulations available for retail sale are expensive if they must be applied in concentrated form (to $100/gallon, depending on dilution and brand). IPAW (2007) emphasizes that stems must be freshly, carefully and cleanly cut, that all surfaces must be treated, and that runoff must be prevented. This becomes tedious on younger shrubs with numerous small stems. It is difficult to expect this level of diligence from paid work crews working on a large project, not to mention volunteers and private individuals.

The objective of this work was to test an alternative honeysuckle control method which seemed more consistent with shrub biology and the attributes of glyphosate herbicide. It was expected that the method would be less expensive and could be more easily implemented by volunteers and private landowners. It emphasizes the best practice of limiting quantities of herbicide applied. The rationale for this approach is based on the ecophysiology of honeysuckle in forest understories.

Invasive bush honeysuckles in deciduous forests typically leaf out several weeks before the tree canopy (Barnes and Cottam 1974). Harrington *et al.* (1989) have shown for *L.* x *bella* that early leaf display significantly enhances carbon gain over closed canopy conditions. Early spring photosynthesis is likely to occur in *L. maackii* as well. We speculated that early canopy development could be exploited to limit energy reserves (storage carbohydrates) available for resprouting, and stimulate the formation of a small volume of replacement foliage amenable to glyphosate spraying. Because spring foliage represents the use of stored carbohydrates to support growth, the period immediately following leaf growth is the time at which whole plant carbohydrate reserves in woody species are at a minimum (Kozlowski and Pallardy 1997). Decapitating the shrub after leafing eliminates any photosynthetic benefit from carbohydrates recently invested in foliage. Additionally, storage reserves to resprout are more limited. Sprouting requires several weeks, which allows the tree canopy to close and limit light availability for understory photosynthesis. Herbicide applied to regrowth can be less concentrated because it is applied to foliage, not woody tissue. Although herbicide overspray is a consideration, regenerated foliage has a far smaller volume and a more compact growth form compared to mature plants.

Two experiments were conducted. The first experiment evaluated whether early growing season cutting treatments might indeed be effective, less chemically intensive control techniques. In this experiment, we also evaluated the success of less concentrated glyphosate applications to stems cut in summer. Because early cutting treatments proved promising, a second experiment was conducted to determine if cutting height (base or at 50 cm) affected the level of control, and verify that herbicide treatment (or other secondary treatment) was necessary for spring cut stems in shaded situations (c.f. Luken and Mattimiro 1991).

METHODS AND MATERIALS

Studies were conducted in a ca. 45 year old upland successional forest dominated by *Robinia pseudoacacia, Acer negundo*, and *Ulmus* spp. on the Southern Illinois University Edwardsville Campus (38° 47' N, 90° 00' W), in Madison County, southwestern Illinois. The honeysuckle population in the stand was entirely *L. maackii* (as is typical of the region). Experimental plants were 1.0-2.5 m tall, although individuals in the population ranged from seedlings to > 3 m tall. The overhead canopy was closed, with midsummer light levels < 3% of ambient photosynthetically active radiation. The soil on the site is moderately acid Menfro Silt loam , containing ca. 22% clay in the surface horizons (Leeper 2004).

Experiment I

In 2004, 80 shrubs were assigned at random to one of four treatments:

1) All stems cut to 30 cm height at the time of maximum leaf expansion (early April). Regrowth sprayed with glyphosate in first week of July.
2) All stems cut to 30 cm height at the time of maximum leaf expansion (early April). Regrowing foliage cut off at mid growing season (first week of July).
3) All stems cut to 30 cm height at mid growing season (first week of July). Coat fresh stubs with glyphosate.
4) All stems cut to 30 cm height at mid growing season (first week of July). No subsequent treatment.

Roundup® (Monsanto, Inc., St. Louis, MO) (18% glyphosate) was mixed with tap water at ca. 2X recommended label recommendations for foliar application and 4X recommended concentration for stump application. These solutions correspond to 0.9 and 1.8% glyphosate. Foliage and stems were sprayed using a pump bottle until uniformly wet. Stems were cut with an ordinary garden loppers.

Treatment success was evaluated in mid November (135 days later) by photographing shrubs against a calibrated background, and scoring the regenerated foliage in index classes corresponding to the area of 5 typical leaves. A double blind procedure was used to prevent observer bias. Shrubs with no foliage were classified as dead. Foliage production classes were converted to ranks and analyzed by 1-way ANOVA and linear contrasts. Mortality rates were compared using contingency table analysis with Chi-square and Fisher's Exact tests (Zar 1999).

Experiment II

In experiment I early cutting with treatment of regrowth proved to be the most effective control technique. We hypothesized that adjusting cutting height might further enhance control. In addition, we speculated that secondary treatment of regrowth might not be necessary in shrubs that are cut at ground level and resprout in deep shade. Experiment II employed a factorial design to evaluate how cutting height and subsequent retreatment (foliar glyphosate application/no treatment) affected control. In addition, light availability and initial shrub size were evaluated as potential confounding influences.

In 2005, 100 shrubs 1.0-2.0 m tall from the same locale as above were assigned at random into four treatment combinations in a 2 X 2 factorial: early cutting (as above) at the

stem base vs. cutting at 50 cm height, and 2X herbicide retreatment of foliage (as detailed above) vs. no retreatment. Before treatment the canopies of all shrubs were measured to determine the lengths of the major and minor axes. These were converted to projected canopy cover. In addition, measurements of photosynthetically active radiation (PAR, photon flux density at λ= 400-700 nm) were made on four clear days in September over each shrub using a 90 cm Canopy Ceptometer (Decagon Devices, Pullman WA). Mean PAR calculated on log transformed measurements were estimated for each shrub. Leaves were harvested, dried at 60 C, and weighed in mid October. Shrubs having no leaves were classified as dead. Treatment effects on foliage production were analyzed using a mixed model incorporating the simple effects height of cutting, herbicide treatment, $\log_{10}$ canopy cover, $\log_{10}$ mean PAR, and 2 way interactions between each pair of simple effects. Foliage production was $\log_{10}$ transformed after adding 1 to stabilize variances. Mortality rates were compared using multiway contingency table analysis and Mantel-Haenszel χ^2 to compare responses aggregated across strata (Systat 2000). The influence of canopy cover and PAR on responses to herbicide treatment were evaluated by logistic regression as implemented in Systat 10 (Systat 2000).

RESULTS

Experiment I

Experimental treatments produced wide differences in the amount of vegetative regrowth and apparent mortality. Values for the regrowth index ranged from 0 to as high as 10, corresponding to about 50 leaves. Apparent mortality rates ranged from 5% in the shrubs cut at midsummer and not treated with herbicide to 75% in the shrubs cut in early summer and treated with herbicide.

ANOVA of the foliage index revealed highly significant differences between treatments ($F_{3,76}$ = 10.84, $P << 0.0001$; Fig. 1a). Time of cutting (early vs. midseason) produced large and significant differences in regrowth ($F_{1,76}$ = 30.24, $P << 0.0001$). Index values for shrubs cut in the early season averaged the equivalent of five leaves, while shrubs cut later in the season had about four times more foliage (Fig. 1a). The two different herbicide treatment schemes (herbicide applied to regrown foliage vs. cut stubs) produced widely different effects ($F_{1,76}$ = 23.87, $P < 0.0001$). Treatment of regrown foliage produced only about 15% of the regrowth seen in the more concentrated stump treatment (Fig. 1a). Mortality rates paralleled rates of regrowth. Sixty-seven percent of plants cut early in the season appeared dead in November, compared to just 10% of the plants cut in midseason (Fisher's Exact Test $P << 0.0001$; Fig. 1b). Herbicide treatments killed a far greater proportion of plants cut early than plants cut later (75 vs. 15%, Fisher's Exact Test $P << 0.0001$; Fig. 1b). Differing followup treatments *within* a given cutting regime had no effect. For shrubs cut in spring the regrowth indices and mortality did not differ significantly whether shrubs were subsequently sprayed or recut (regrowth index: Fig. 1a, $F_{1,76}$ = 1.89, P = 0.1729; mortality: Fig. 1b, Fisher's Exact P = 0.5006). Shrubs cut in midseason did not respond to herbicide treatment. The regrowth index for herbicide/no herbicide was ca. 20 leaves ($F_{1,76}$ = 0.38, P = 0.5385, Fig. 1a); mortality was about 10% (Fisher's Exact P = 0.6049, Fig. 1b).

Experiment II

Manipulations caused large differences in end of season leaf biomass and apparent mortality. Leaf biomass ranged from 0 to 8.2 g; mortality was lowest (4%) in shrubs cut at the base and not treated with herbicide and greatest (85%) in shrubs cut at the base and treated with herbicide. Median shrub canopy cover was 3.8 m^2, but ranged widely, 0.2 to 20.9 m^2. Median average PAR for individual shrubs was 30 μmol m^{-2} s^{-1}, ranging 3-1159 μmol m^{-2} s^{-1}.

ANCOVA of $\log_{10}$ leaf mass produced unambiguous results, identifying herbicide application as the single statistically significant determinant of regrowth (Table 1). Least squares means, controlling for all other main effects and the interactions named above, show that the average shrub receiving herbicide treatment produced only 0.33 g leaf tissue compared to 1.74 g produced by untreated shrubs (Fig. 2a). Canopy cover had a statistically ambiguous positive influence on regrowth (Table 1). Notably, light availability and shrub canopy cover did not modify treatment responses: all interactions are nonsignificant (Table 1).

Contingency table analysis showed that cutting height had no effect on mortality (Mantel-Haenszel $\chi^2 = 0.10$, $P = 0.7500$; Fig. 2b). Herbicide application had an important effect, killing 76% of treated shrubs as compared to 8% mortality in untreated shrubs (Mantel-Haenszel $\chi^2 = 44.01$, $P << 0.0001$; Fig. 2b). There were no interactions between cutting height and herbicide treatment (Mantel-Haenszel $\chi^2 = 0.12$, $P = 0.8888$; Fig. 2b).

A limited logistic regression analysis was conducted to detect effects of canopy cover and light availability on responses to herbicide treatment. Neither canopy cover nor light availability had significant effects by themselves, or in interaction with herbicide treatment. Herbicide treatment was significant in both models (Table 2).

DISCUSSION

Early spring cutting and spraying of regrowth with 0.9% glyphosate (the "new technique") produced results comparable to reported results for midseason cutting and stump treatment with 20% glyphosate (Kline 1981). For the new technique the mortality rate over two seasons was 80.4% (s.e. = 5.9%). Kline (1981) killed 89.0% (estimated s.e. = 2.7% based on sample size) of shrubs using a 1:5 glyphosate dilution, which might slightly exceed the new technique ($P = 0.0681$). The new technique has the advantage of requiring less herbicide. We estimate that spraying requires about double the volume of herbicide solution, but at 1/10th the concentration is a four fold reduction in herbicide use.

In the first experiment regrowth after early cutting was subsequently sprayed or cut off. On a statistical basis, both secondary treatments had indistinguishable effects, indicating that recutting may be a practical alternative to herbicide. Luken and Mattimiro (1991) suggested that repeated cutting of regrowth showed potential as a control technique in shaded sites. In their study it required four years of cutting in midseason to achieve significant control. Early cutting, as executed here, followed by a few cycles of cutting regrowth may be more effective than Luken and Mattimiro's approach. Treatment schemes that avoid herbicide application are particularly desirable. Although this project

was undertaken in a very low grade forest with a flora of no particular conservation value, most managers are apt to practice triage, and focus honeysuckle extermination efforts on high quality forests where richer floras are at risk. Recent concerns over losses of herpetofauna and the effects of Roundup® on amphibians (Relyea 2005a,b) should also motivate additional investigations.

The fact that both herbicide application or cutting of regrowth are effective on shrubs cut in early spring supports the proposal that the seasonal distribution of carbohydrates is a major vulnerability in honeysuckle. Based on the results of experiment I, we speculated that one carefully scheduled cutting alone might kill many shrubs by imposing a limited pool of carbohydrate reserves after leaf flush, and the loss of photosynthetic opportunity (lost leaf surface and reduced light availability with canopy closure). Experiment II showed that this speculation was incorrect. Spring cutting without subsequent treatment killed just 12.5% of shrubs. This does not differ meaningfully from 5% mortality after midseason cutting and no secondary treatment in experiment I.

Different cutting heights were evaluated because honeysuckle resprouts from dormant buds on the stems and root crown. It was suspected that cutting at 50 cm would stimulate more abundant regrowth because more dormant buds remained. More extensive regrowth would deplete storage carbohydrates more thoroughly, and provide greater surface for herbicide absorption. Cutting height had absolutely no effect on regrowth or mortality of either herbicide treated or untreated shrubs. From the standpoint of best practices, cutting at the base is preferable because herbicide overspray is apt to be less.

We found limited indication that shrub size, measured by canopy spread, affected treatment response. The ANCOVA of leaf biomass showed a weak ($P = 0.0860$) response to $\log_{10}$ canopy cover, but the interaction between herbicide application and canopy cover did not influence mortality (Table 2). A study with larger sample size and greater statistical power would likely show that larger shrubs do not succumb as easily as small shrubs. It is not clear whether size might affect treatment success for some physiological reason, or merely because secondary treatment was less uniform on a larger shrub.

Light availability expressed as $\log_{10}$ PAR had no effect on response. This finding must be interpreted in the context of the wide range of light regimes inhabited by honeysuckle, and the narrower range studied here. Shrubs in the forest interior are significantly light limited to varying degrees. Often honeysuckle inhabits open habitats in which light limitations are far less pronounced. In the shade, regrown shrubs always encountered a significant, albeit probably variable, limitation on photosynthesis. It is a reasonable hypothesis that early cutting and herbicide treatment would be less effective in brighter habitats because new foliage could achieve sustained periods of high photosynthetic rate and support regrowth more easily.

This study provided evidence that early cutting and foliar herbicide application is an effective means to control bush honeysuckles in forest understories. It has the advantage of greatly reducing the total quantity of herbicide applied, but does not produce better results than the traditional approach. Notably, these results refute the assertion that treatment in spring is ineffective. Rather, herbicide application *to stumps* may be ineffective in spring.

Spring treatment has advantages over other times of the year. Early leaf display makes honeysuckle shrubs visible against a background of dormant native species. Cutting also removes the competitive effect of the canopy before the growing season, and stimulates regrowth that is a visible, compact, and physiologically active target for herbicide action. For the private landowner early cutting and retreatment is desirable because herbicide application is less painstaking. Small quantities of properly diluted glyphosate can be purchased in spray bottles for easy application. Moreover, work conditions are more temperate early in the growing season. In forests with significant summer green flora, foot traffic and herbicide spraying need to be carefully controlled when regrowth is treated.

Both the new and traditional methods are considerably more labor-intensive, which can become costly. The traditional method could be the only choice if work crews are otherwise occupied during spring. Invasives campaigns often take place in fall and winter because crews are available. If it is possible to assemble a sufficient number of laborers, the new method proposed here is certainly effective, and expands the time window for restoration work.

SUMMARY

Asiatic shrub honeysuckles (*Lonicera* spp.) are aggressive invaders of forest and forest edge habitats throughout the Midwest. Control methods for these species are not widely described or evaluated in the open literature. Kline's (1981) method of stem cutting followed by application of 20% glyphosate to the stumps remains widely recommended despite the fact that glyphosate is most effective as a foliar spray as opposed to a stump treatment. We developed and evaluated a cutting and herbicide technique that capitalizes on the normal flux of storage carbohydrates in woody understory plants. For forest understory honeysuckles, stem cutting in spring just after leaf expansion removes foliage before meaningful levels of photosynthesis occur, and probably inflicts a significant cost on the shrubs carbohydrate reserves. Retreatment of regrowth about eight weeks later, either by recutting or spraying with modest concentrations of glyphosate, killed about 80% of treated shrubs. Applications of an approximately equivalent amount of glyphosate to cut stems was ineffective. The technique described here is simpler and cheaper for small organizations and private landowners to implement.

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LITERATURE CITED

Abrams, M.C. and S.A. Mostoller. 1995. Gas exchange, leaf structure and nitrogen in contrasting successional tree species growing in open and understory species during a drought. Tree Physiology 15: 361-370.

Barnes, W.J. and G. Cottam. 1974. Some autecological studies of the *Lonicera* x *bella* complex. Ecology 55: 40-50.

Batcher, M.S. and S.A. Stiles. 2000. Element stewardship abstract for *Lonicera maackii* (Rupr.) Maxim (Amur honeysuckle), *Lonicera morrowii* A. Gray (Morrow's honeysuckle), *Lonicera*

tatarica L. (Tatarian honeysuckle), *Lonicera* x *bella* Zabel (Bell's honeysuckle) the bush honeysuckles. The Nature Conservancy, Arlington, Virginia. http://tncweeds.ucdavis.edu/esadocs/documnts/loni_sp.pdf. Last access, October 22, 2007.

Collier, M.H. J.L. Vankat and M.R. Hughes. 2002. Diminished plant richness and abundance below *Lonicera maackii*, an invasive shrub. American Midland Naturalist 147: 60-71.

Gould, A.M. and D.L. Gorchov. 2000. Effects of the invasive shrub *Lonicera maackii*, Amur honeysuckle, on native forest annual herbs. American Midland Naturalist 144:36-50.

Harrington, R.A., B.J. Brown, and P.B. Reich. 1989. Ecophysiology of exotic and native shrubs in southern Wisconsin USA; relationship of leaf characteristics, resource availability, and phenology to seasonal patterns of carbon gain. Oecologia 80: 356-367.

Hartman, K.M, and B.C. McCarthy. 2004. Restoration of a forest understory after the removal of an invasive shrub, Amur honeysuckle (*Lonicera maackii*). Restoration Ecology 12: 154-165.

Ingold, J.I. and M.J. Craycraft. 1983. Avian frugivory on honeysuckle (*Lonicera*) in southwestern Ohio in fall. Ohio Journal of Science 83: 256-258.

IPAW (Invasive Plants Association of Wisconsin). 2007. Honeysuckle identification and control. http://IPAW.org. Last access: June 14, 2007.

Kline, V. 1981. Control of honeysuckle and buckthorn in oak forests. Restoration and Management Notes 1:18.

Kozlowski, T. T. and S. G. Pallardy, 1997. Physiology of Woody Plants. Academic Press, San Diego.

Leeper, R.A. 2004. Soil Survey of Madison County, Illinois. United States Department of Agriculture, Natural Resources Conservation Service and Illinois Agricultural Experiment Station. http://soildatamart.nrcs.usda.gov/Manuscripts/IL119/0/madison_IL.pdf. Last access: June 6, 2007.

Love, J.P., J. A. Edalgo, and J.T. Anderson. 2006. Management plan for a degraded meadow infested with Morrow's honeysuckle. West Virginia University Division of Forestry and Natural Resources, Wildlife and Fisheries Resources Program. http://www.forestry.caf.wvu.edu/jAnderson/FONE_manage_plan.pdf. Last access: June 11, 2008.

Luken, J.O. and N. Goessling. 1995. Seedling distribution and potential persistence of the exotic shrub *Lonicera maackii* in fragmented forests. American Midland Naturalist 133: 124-130.

Luken, J.O. and D.T. Mattimiro. 1991. Habitat-specific resilience of the invasive shrub Amur honeysuckle (*Lonicera maackii*) during repeated clipping. Ecological Applications 1: 104-109.

McNabb, K. 1996. Forestry herbicide facts. ANR-629 Alabama Cooperative Extension System. http://www.aces.edu/pubs/docs/A/ANR-0629. Last access, October 22, 2007.

Missouri Conservation Commission. 2007. Curse of the bush honeysuckles. Missouri Department of Conservation, Jefferson City.

Monsanto. 2006. Materials safety data sheet for Roundup Original MAX® herbicide. Monsanto, Co., St. Louis, MO. http://www.monsanto.com/monsanto/ag_products/pdf/labels_msds/roundup_orig_max_msds.pdf. Last access October 22, 2007.

NCDC (National Climatic Data Center). 2007. Climatography of the United States no. 85 divisional normals and standard deviations of temperature, precipitation, and heating and cooling degree days 1971 – 2000 (and previous normals periods). http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/ climatenormals.pl. Last access October 22, 2007.

Nyboer, R. 1992. Vegetation management guideline: bush honeysuckles – Tatarian, Morrow's, belle, and Amur honeysuckle (*Lonicera tatarica*, L., *L. morrowii* Gray, *L.* x *bella* Zabel, and *L. maackii* [Rupr.] Maxim.). Natural Areas Journal 12: 218-219.

Relyea, R.A. 2005a. The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecological Applications 15: 1118-1124.

Relyea, R.A. 2005b. The lethal impact of Roundup and predatory stress on six species of North American tadpoles. Archive of Environmental Contamination and Toxicology 48: 351-357.

Schmidt, K.A. and C.J. Whelan. 1999. Effects of exotic *Lonicera* and *Rhamnus* on songbird nest predation. Conservation Biology 13: 1502-1506.

Shaner, D. 2006. An overview of glyphosate mode of action: why is it such a great herbicide? North Central Weed Science Society Proceedings Abstracts 61:94. http://ncwss.org/proceed/2006/abstracts/94.pdf. Last access October 22, 2007.

Systat. 2000. Systat version 10. SPSS, Inc., Chicago.

White, D.W. and E.W. Stiles. 1992. Bird dispersal of fruits of species introduced into eastern North America. Canadian Journal of Botany 70: 1689-1696.
Woods, K. D. 1993. Effects of invasion by *Lonicera tatarica* L. on herbs and trees in four New England forests. American Midland Naturalist 130: 62 74.
Zar, J.H. 1999. Biostatistical analysis 4th ed. Prentice Hall, Upper Saddle River, NJ.

Table 1. ANCOVA of leaf mass (transformed as $\log_{10}(X + 1)$) remaining at the end of the growing season after manipulations of cutting height and herbicide followup treatment. PAR (photosynthetically active radiation) and projected canopy area were evaluated as influences on treatment response.

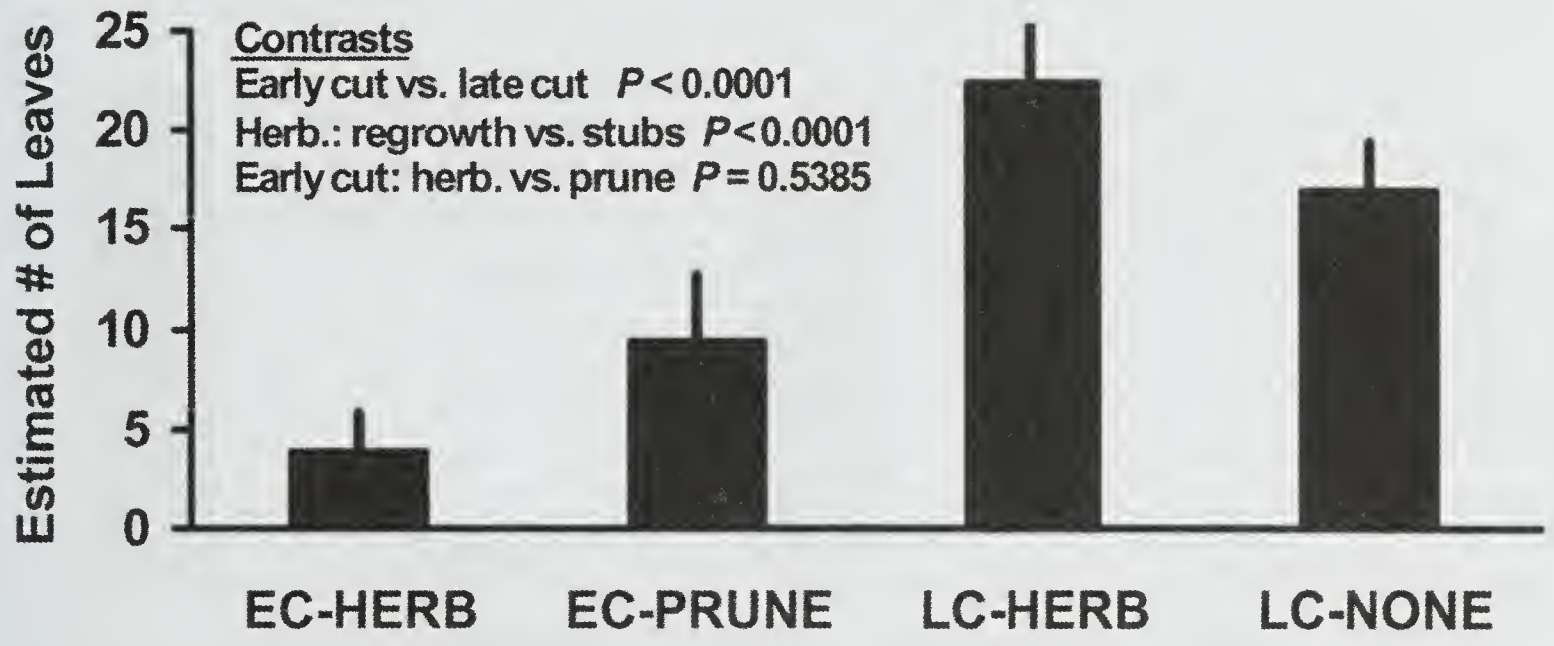
Source	Sum of Squares	Df	Mean Square	F	*P*
Cutting height	0.1136	1	0.1136	0.06	0.8010
Herbicide	7.9933	1	7.9933	4.50	0.0371
$\log_{10}$(PAR)	0.7639	1	0.7639	0.43	0.5139
$\log_{10}$(canopy area)	3.8488	1	3.8488	2.17	0.1451
Cutting height X herbicide	1.1403	1	1.1403	0.64	0.4254
Cutting height X $\log_{10}$(PAR)	0.7165	1	0.7165	0.40	0.5272
Herbicide X $\log_{10}$(PAR)	1.5153	1	1.5153	0.85	0.3585
Cutting height*$\log_{10}$(canopy cover)	0.1228	1	0.1228	0.07	0.7933
Herbicide*$\log_{10}$(canopy cover)	3.5318	1	3.5318	1.99	0.1625
Residual	134.9418	76	1.77555		

Table 2. Logistic regression of mortality rate as affected by followup herbicide treatment, photosynthetically active radiation (PAR), and projected canopy area.

Estimate	Estimate	S.E.	t	*P*	Odds ratio
Cutting height	-2.28	0.53	-4.34	0.0000	
Herbicide application	5.69	2.70	2.11	0.0351	296.8
Herbicide application X $\log_{10}$(canopy cover)	-1.38	1.46	-0.95	0.3445	0.3
Herbicide application X $\log_{10}$(PAR)	-0.21	1.41	-0.15	0.8802	0.8
LLratio for full model = 67.098147, df = 3, $P < 0.0001$					

Figure 1 a, b. Responses to spring and fall stem cutting with followup treatments. a. Estimated numbers of leaves based on leaf abundance index ($\bar{x}$ and s.e.). b. Apparent rate of mortality (proportion and s.e.). Treatment identifiers: EC-HERB, cut early April/herbicide spray early July; EC-PRUNE, cut early April/prune off regrowth early July; LC-HERB, cut early July, spray stumps; LC-NONE, cut early July, no other treatment.

1a.



1b.

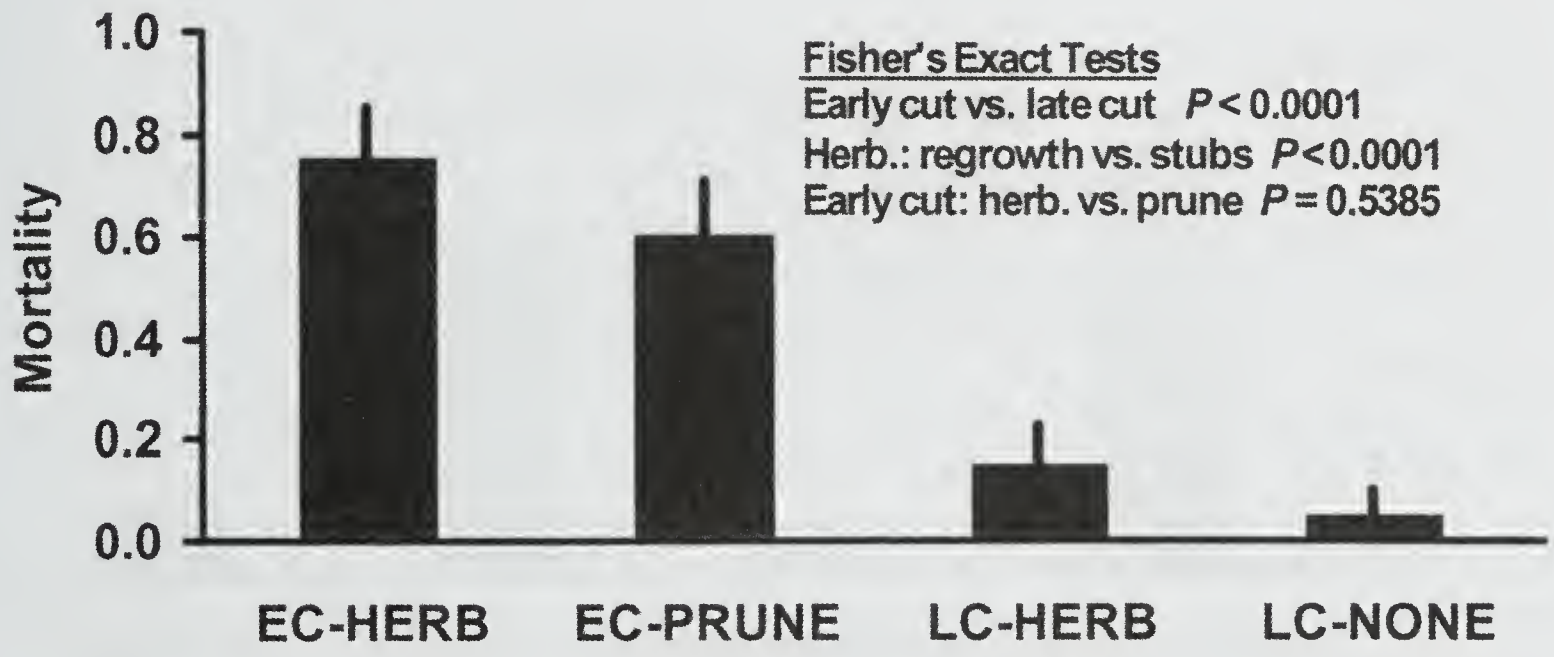
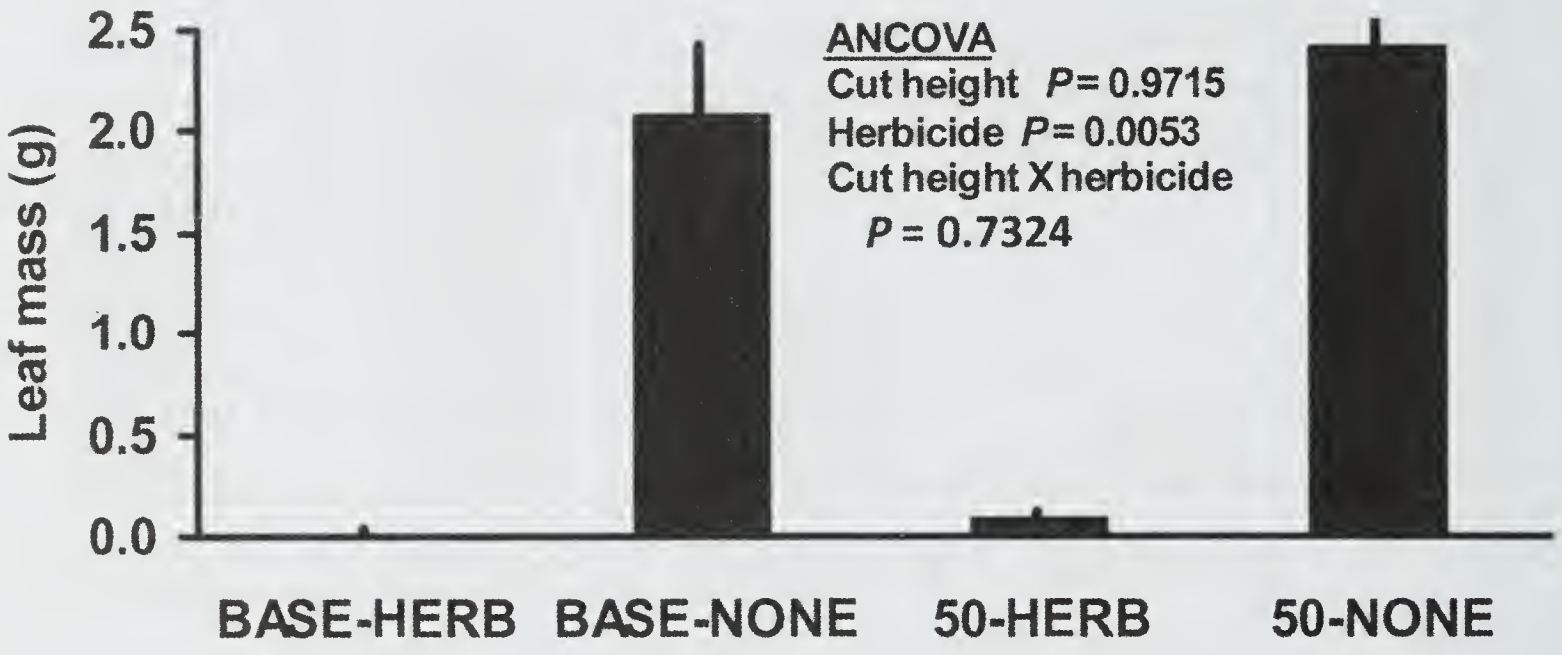
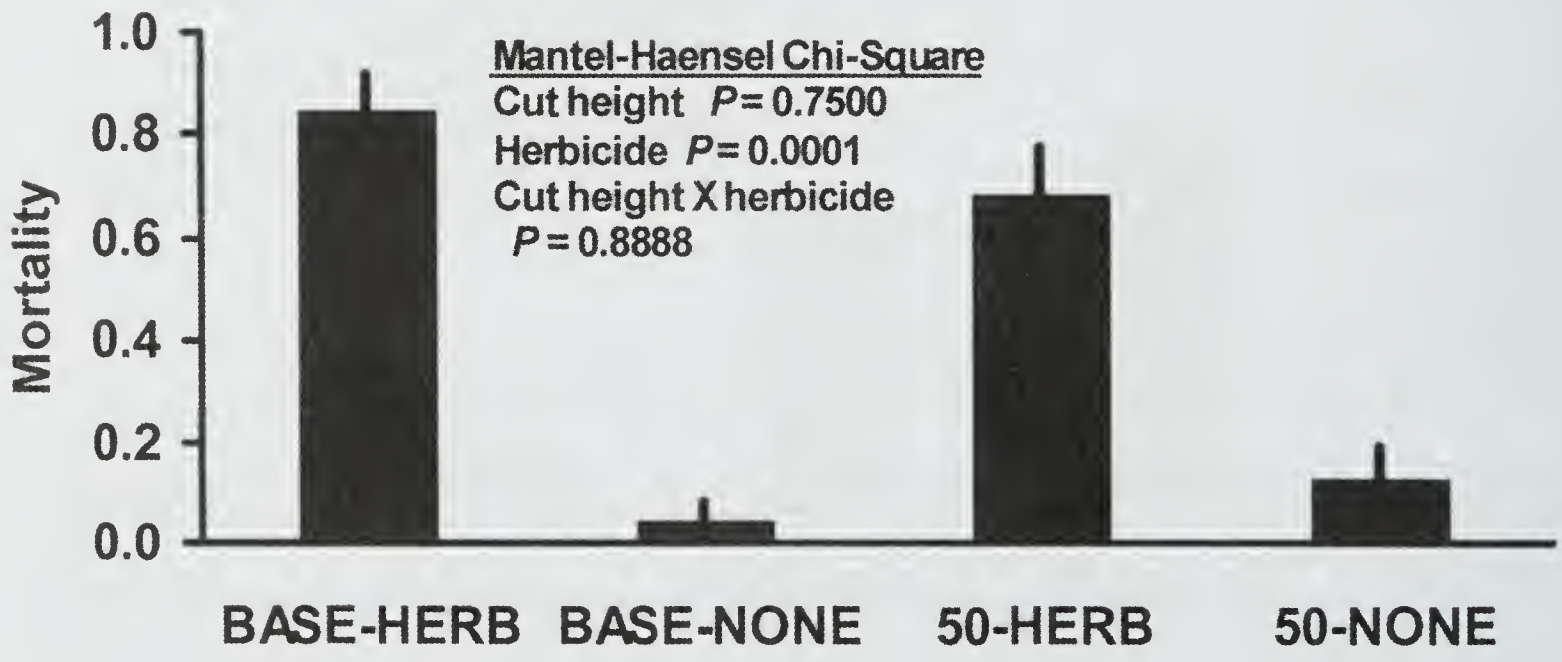


Figure 2 a, b. Responses to height of spring (early April) spring stem cutting and herbicide treatment. a. Dry weight of leaves in October ($\bar{x}$ and s.e.). b. Apparent rate of mortality (proportion and s.e.). Treatment identifiers: BASE-HERB, cut at base/herbicide spray regrowth early July; BASE-NONE, cut at base, no other treatment; 50-HERB, cut at 50 cm, spray regrowth early July; 50-NONE, cut at 50 cm, no other treatment.

2a.



2b.



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Induction of the Macrostomal Form of *Tetrahymena vorax* by a Synthetic Ferrous Iron Chelate of Hypoxanthine and Uracil

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ABSTRACT

Tetrahymena vorax is a polymorphic ciliate capable of forming two cell types that depend on environmental cues. One type, called the microstomal form, feeds on bacteria and other small particulate matter. The other, the macrostomal cell, is a carnivore and feeds on small ciliates or flagellates. Large numbers of synchronously differentiating macrostomal cells can be induced by suspending microstomal cells in stomatin, an exudate produced by a potential ciliate prey, *Tetrahymena pyriformis*. The active principle in stomatin has been identified as a ferrous iron chelate of hypoxanthine and uracil (Smith-Somerville et al., 2000). They produced a synthetically derived chelate with strong biological activity equivalent to that of stomatin. The purpose of our study is to use *our* chemicals and *our* strain of *Tetrahymena vorax*, and following the methods of Acevedo-Chavez et al., 1996 as modified by Smith-Somerville et al., 2000 to synthesize an active chelate. Our results show that while lower levels of chelate (1, 5, and 10 ug/ml) had little or no effect on levels of differentiation, chelate concentrations in the range of 100 ug/ml induced macrostomal differentiation equivalent to 78 % of the stomatin control value. Our results confirm findings published by Smith-Somerville et al. 2000.

Key words: autophagic vesicle, differentiation, iron chelate, macrostome, microstome, stomatin.

INTRODUCTION

Tetrahymena vorax is a fresh water polymorphic ciliated protist. One form, termed the microstome (Fig. 1), feeds by accumulating bacteria and small particulate matter in phagosomes that form successively at the base of the oral apparatus. Under the right circumstances, the microstomal form differentiates into large carnivorous cells called macrostomes (Fig. 2) that is capable of feeding on small ciliates and flagellates (Buhse, 1966). In addition to the larger oral apparatus, the macrostomal form is equipped with a

large pouch into which prey is engulfed and processed by digestion. Differentiation to the macrostomal form is triggered by stomatin (Buhse, 1967), a collection of catabolic products released by a potential prey, *Tetrahymena pyriformis* (Buhse, 1965). Stomatin is heat stable, is most effective at acidic pH values, and exhibits a pale yellow color in solution (Buhse, 1966). A bioassay has been developed to test the inductive qualities of stomatin preparations. In this assay 70-90 % of microstomal cells are induced to differentiate synchronously into the macrostomal phenotype 6 to 8 hr following addition of 3 mg/ml stomatin (final concentration) (Buhse, 1967).

Recently, the active principle that initiates microstomal to macrostomal differentiation has been identified as an ferrous iron chelate of hypoxanthine (6 hydroxy-purine) and uracil (2,4-dioxyprymidine) (Smith-Somerville et al., 2000). Earlier studies by LeBoy, et al., 1967, showed that nucleotide catabolic products including hypoxanthine and uracil were excreted by *Tetrahymena pyriformis*. As pointed out by Ryals et al., 2002, it is not known whether the chelate is assembled before or after excretion or what role, if any, the complex has in iron transport or metabolism. The chelate, a paracrine released by a potential prey, is postulated to act as a ligand binding to the surface of the undifferentiated microstomal form and activating a signal transduction system leading to macrostomal differentiation (Ryals et al., 1999). A biologically active iron chelate has been synthesized using the methods of Acevedo-Chavez et al., 1996 as modified by Smith-Somerville et al., 2000. Addition of 50 ug/ml of chelate to microstomal cells induced macrostomal differentiation at levels equivalent to the stomatin control (Smith-Somerville et al., 2000). The synthetic chelate exhibits many of the same characteristics as stomatin: it is heat stable, a property of chelates (Ueno et al., 1992), active at acid pH values, and is a pale yellow color in solution. A review of *Tetrahymena vorax*, including the macrostomal induction system, has been published (Ryals et al., 2002)

The purpose of this study is to synthesize the iron chelate using *our* chemicals and test its activity against *our* strain of *Tetrahymena vorax* using the stomatin bioassay system for macrostomal induction. Positive results from these experiments will serve as a prelude to further studies aimed at delineating the stoichiometrics of the active complex. It is postulated that only certain configurations of the chelate induce macrostomal differentiation (Ryals et al., 2002).

MATERIALS AND METHODS

Cell maintenance

Tetrahymena vorax microstomal cells and *T. pyriformis* cells were grown in 50 ml of Loefer's medium [15 g proteose-peptone, 5 g bacto-casitone, 5 g glucose, 2 g NaCl, 1 g NaH_2PO_4, 1 g K_2HPO_4, 0.5 g yeast extract] (Loefer et al., 1935) contained in 500 ml Erlenmeyer flasks. Cells were grown at 20°C and sub-cultured every two days (10 mls of culture to fresh medium) following methods of Buhse, 1967.

Prepartion of stomatin

Stomatin preparation was carried out following Buhse, 1967. Fifty mls of a two-day old culture of *T. pyriformis* cells was transferred to 1 liter of Loefer's medium contained in 2.8-liter low form flasks. The inoculated cells were grown for two days (48 hr) at room temperature with moderate shaking. At the end of this time, the cells were removed by

centrifugation at 1500 RPM in a Sorvall centrifuge (model RC-5B) equipped with GSA rotor capable of holding six 250 ml plastic bottles. The concentrated cells were washed 3 times by centrifugation, suspended in a final rinse (100 ml) of double distilled water, and placed in a Roux flask over night at 20°C. The next day, the cells were removed by centrifugation (1500 RPM) in 50 ml conical centrifuge tubes using a IEC HN-2 table top centrifuge (rotor radius 10 cm). The resultant "conditioned media" was centrifuged twice more in a Sorvall RC-5B at 5000 RPM using the SS34 rotor. The cell-free liquid was concentrated by roto-evaporation to dryness, weighed, re-suspended in double distilled water, filtered using a Nalgene 0.5 um filter attached to a 10 ml syringe, and diluted to yield a final concentration of 6 mg/ml stomatin. The stomatin preparation was dispensed into 1-ml volumes (6 mg/ml) and stored frozen at minus 20°C .

Scanning Electron Microscopy

Tetrahymena cells were washed twice in $2.49 X10^{-4}$M sodium phosphate and $7.04 X10^{-4}$M magnesium sulfate buffer at pH 7.0. They were then pelleted and resuspended in 600 ul of wash buffer, 5 ul IGSS gelatin (Amersham Biosciences), 40 ug BSA (Sigma), 1.25 ul Tween 20 (Sigma), and 50 ul Sodium Azide (Sigma) and brought to volume of 5 mls. They were fixed by adding 200 ul of 2.5 % gluteraldhyde and 200 ul of a 1% osmium tetraoxide each of which was suspended in the sodium/magnesium buffer at pH 7.0. The cells and fixing solutions were mixed thoroughly, placed in a tray of ice, and microwaved (Pelco Biowave) for 30 seconds at power level 1(100 % power). The microwaved cells were left on ice for 25 min, then washed twice for 5 min each in a buffer. They were then suspended in a graded ethanol series of 10, 25, 40, 50, 65, 75, 85, and 95% followed by three washes in 100 % ethanol for 5 minutes each. A drop of the dehydrated cells was placed on a poly-L-lysine coated coverslip and critical-point dried (Balzers CPD030 critical point dryer). The dried samples were then mounted on an aluminum specimen stub with double sticky tape, coated with 15nm gold (Anatech Hummer Jr. sputter coater), and viewed using a JEOL 5600LV scanning electron microscope.

Preparation of the iron chelate

The iron chelate was prepared by the methods of Acevedo-Chavez et al., 1966, as modified by Smith-Somerville et al, 2000. Briefly, 1 mM hypoxanthine, 1 mM uracil, and 10 ml of 2 mM ferrous sulfate solution was added to 200 ml of a glycine-HCl buffer (0.084 g glycine, 0.66 g NaCl and 1.87 g HCl made up in 500 ml). The chelate solution (pH 1.5) was air dried by stirring slowly for two weeks at room temperature. The resultant residue was washed twice in cold (4°C) double distilled water and oven dried at 50°C for 2 days. Seven mg of dried chelate was dissolved in 7 ml of inorganic medium, IM (1 g Na_2HPO_4, 1 g KH_2PO_4, and 0.3 g $MgSO_4$ per liter) and the pH was adjusted to 5.7 by addition of dried Na_2HPO4. This preparation was then divided into seven 1ml samples and frozen at minus 20°C.

Experimental design

For these experiments, *T. vorax* microstomal cells were washed three times in inorganic medium and suspended in one half ml of inorganic medium contained in 15 x 1 cm Kimax culture tubes. The cells were exposed to several chemical compounds that also were dissolved in one half-ml aliquots. **(1)** Inorganic medium at pH 5.7 functioned as the negative control. **(2)** Three mg/ml stomatin final concentration functioned as the positive control (Buhse, 1967). **(3)** Various concentrations of the synthetically derived chelate (1,

5, 10, 50 and 100 ug/m final concentration) were added to the remaining culture tubes containing the cells and incubated at 20°C for 8 hr before being killed. The percentage of macrostomal differentiation in each tube was determined microscopically (Buhse,1967).

RESULTS

We determined the morphological effects of the chelate on treated populations and compared them to the negative (IM) and positive (stomatin) controls. Differences between the microstomal and macrostomal cells are easily seen in the photomicrographs (Figs. 1 & 2). The microstomal cells are elongate and small, while the macrostomal cells are larger and contain the prey receptacle which occupies over 80 % of the cell volume. Fig. 3 shows results from experiments placing microstomal cells in inorganic medium. There are very few macrostomal cells (arrows). The similar results were obtained for cells suspended in 1 ug /ml chelate, shown in Fig. 4. Three mg /ml stomatin (Fig. 5) and 100 ug/ml the chelate (Fig. 6) induce comparably higher levels of macrostomal cells. However, the levels of macrostomal induction were consistently lower than the stomatin control and values reported by Smith-Somerville et al. 2000 for comparable levels of chelate.

Next, we quantified our data by determining the percentage of macrostomal cells induced by each treatment. The data depicted in Table I show two experiments using different concentrations of the synthetic iron chelate of hypoxanthine and uracil. For trial 1, we dissolved 1000 ug of chelate in 1 ml then diluted this stock solution to 200 ug/ml. This stock was further diluted to give a final concentration of 100, 50, 10, 5, and 1 ug/ml after mixing 1:1 with washed microstomal cells. While determining the percentage of macrostomal cells, we noticed some of the chelate particles hadn't dissolved which may help to explain why we attained lower percentages of macrostomal induction than Smith-Somerville et al., 2000. In the dry state, the chelate appeared as a greenish powder, which dissolved completely prior to freezing. It is possible that upon freezing at this concentration some of the chelate precipitated. When we conducted the second experiment (shown as trial two), we dissolved 500 ug of chelate in 1 ml and diluted this stock to give the five chelate concentrations listed in Table I. Using this method, the chelate was dissolved completely and no particles were seen following thawing of the chelate. In each case, the percentage of macrostomal differentiation induced by stomatin was slightly more than 80 % while the negative control values hovered around 5 to 6 %. Table I shows (from left to right) the average results from trial 1 and 2 separately, and the combined average for the two experiments. The treatments were replicated three to five times (N) within each sample set and the experiment was repeated twice using the same batch of chelate. The standard error of the mean ($sigma_x$) was calculated for each chelate concentration tested. Finally, each treatment was normalized against the stomatin control that was arbitrarily set at 100 % differentiation. High chelate levels (50 or 100 ug/ml) of synthetically derived iron chelate promote macrostomal differentiation whereas lower levels (1 or 10 ug/ml) did not promote much macrostomal differentiation. While there is a fair amount of variability between experiments as evidenced by the relatively high standard error, it is clear that as the chelate concentration is increased progressively higher levels of differentiation are obtained culminating in 57.7 and 78.4 % of the stomatin control for 50 and 100 ug/ml, respectively.

DISCUSSION

Our findings show that the synthetically derived chelate induces levels of macrostomal differentiation within the range of the stomatin controls. The level of differentiation is positively correlated with the concentration of the chelate. Higher quantities (50 and 100 ug/ml) induce about 60 % and 80 % of stomatin controls, respectively, whereas lower quantities of chelate elicit levels of differentiation equivalent to that of cells suspended in inorganic medium. Our results are comparable with previous results (Smith-Somerville et al., 2000) that showed higher levels of chelate induced higher levels of macrostomal differentiation. In their case, 50 ug/ml induced 90 % of stomatin controls. For our experiments, 50 ug of chelate induced slightly less than 60 % of control values. These differences can be explained by the following or a combination of the following arguments as they are not mutually exclusive and may in fact be acting synergistically. Smith-Somerville et al., 2000 grew *T. vorax* microstomal cells on an iron containing medium prior to exposing them to the synthetic chelate, whereas we grew cells on Loefer's medium. They carried out their experiments at pH 5.0 while our experiments were run at pH 5.7. It has been shown that the iron chelate is most active at lower pH values than higher pH levels (Ryals et al., 2002). Thus, it is possible that at the higher pH value some of the active ferrous iron chelate was dissociated by oxidization to the ferric form resulting in a decrease of specific activity of our preparation. This conclusion may be directly tested by buffering our chelate to pH 5.0 to determine if there is a significant increase in activity. Another difference between our studies and those of Smith-Somerville et al, 2000 is that in their study the chelate was stored dry and dissolved just prior to use whereas ours was frozen immediately then thawed prior to use. One possibility is that freezing and thawing may have caused particles to precipitate (see Fig. 6) thus lowering the absolute concentration of the chelate. Finally, it is also possible that chelate preparations differ with respect to their ability to induce macrostomal differentiation. The stoichiometry of the iron chelate is not known and several arrangements could be formed. Thus, it is possible that some of these support formation of the macrostomal forms, while others support less differentiation (Ryals et al., 2002). The overall specific activity of the preparation would depend on this mixture. Therefore, and because of this, the level of activity of each batch of chelate may differ. The fact that doubling the concentration of chelate from 50 to 100 ug/ml leads to a significant increase in differentiation from 52 % to 70 % of the stomatin control supports this conclusion.

We plan further studies using the synthetic chelate approach. Using X-Ray diffraction it should be possible to determine the stoichiometry of various elements of the chelate. In anticipation of these studies, we present two possible structures of the chelate (Fig. 7). This model is consistent with coordination potential between hypoxanthine and uracil, however, several other structures are possible.

The question of when and where the chelate is formed remains to be resolved. One possibility is that the chelate forms as a by-product of autophagic vesicle activity. Autophagic vesicles are responsible for degradation of organelles and their formation is triggered by a variety of stresses including starvation (Reggiori & Klionsky, 2000; Mizushima 2007). Once produced, the digested end-products of autophagy including amino acids, proteins, fatty acids, and nucleic acids catabolic products are further hydrolyzed in the mitochondria to produce energy (Levine and Yaun, 2005).

Tetrahymena pyriformis cells form vesicles when they cease growing in organic medium as they enter stationary phase, a stage of growth. Cells enter stationary phase growth because of starvation, and/or oxygen depravation, and/or build up of toxic compounds due to metabolism of large numbers of cells maintained in batch culture within a confined space (Geise, 1962). Evidence that these vesicles are autophagic vesicles include the following observations: they are bounded by a double membrane that contains partially digested organelles and they bind a specific autophagic membrane antibody (data not shown).

We propose that these vesicles facilitate chelate formation because of their extreme internal conditions including acidic pH levels that are achieved following their fusion with lysosomes. The conditions within these vesicle are similar to those described for the production of the synthetic chelate (Smith-Somerville et al, 2000) that also requires reducing conditions and low pH values. We postulate that the small molecules formed and released from the vesicles including the chelate may easily diffuse out of the cell into the suspending medium. Once it is released from the cells, the chelate functions as a paracrine ligand that induces microstomal to macrostomal differentiation in *Tetrahymena vorax*. If this hypothesis is true, it should be possible to prevent stomatin production by blocking autophagic vesicle formation by use of inhibitors such as wortmannin or by knocking out genes that control autophagy specifically.

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LITERATURE CITED

Acevedo-Chavez, R., Costas, M.E., Escudero, R. 1996. Allopurine and hypoxanthine copper II-spectral and magnetic studies of novel dinuclear coordination compounds with bridging

Buhse, H. E., Jr. 1965. Some properties of a substance (s) produced by *Tetrahymena pyriformis* HS which causes microstome-macrostome transformation in *Tetrahymena vorax* V_2. The second International Congress on Protozoology, International Congress series no. 91. Excerpta Medica Foundation p. 233.

Buhse, H. E., Jr. 1966. An analysis of macrostomal production in *Tetrahymena vorax* V_2S-type. J. Protozool., 13: 429-435.

Buhse, H. E, Jr. 1967. Microstome-macrostome transformation in *Tetrahymena vorax* strain V_2S induced by the transforming principle, stomatin. J. Protozool., 14: 608-614.

Giese, A. C. 1962. Cell Physiology. W. B Saunders. Philadelphis, London.. p. 518.

Leboy, P. S., Cline, S. G. and Conner, R. L. 1964. Phosphate, purine and pyrimidine as excretory products of *Tetrahymena*. J. Protozool., 11: 217-222.

Levine, B. and Yuan, J. 2005. Autophagy in cell death: an innocent convict? J. Clin. Invest., 115: 2679-2688.

Loefer, J. B., Owen, R. D. and Christensen, E. 1958. Serological types among thirty-one strains of the ciliated protozoan, *Tetrahymena pyriformis*. J. Protozool., 5: 209-217.

Mizushima, N. 2007. Autophagy: process and function. Genes and Development 21: 2861-2837

Reggiori, F. and Klionsky, D. 2002. Autophagy in the Eukaryotic Cell. Eukaryotic Cell, 11-21.

Ryals, P. E., Bae, S., and Patterson, C. E. 1999. Evidence for early signaling events in stomatin-induced differentiation of *Tetrahymena vorax*. J. Eukaryot. Microbiol., 46:77-83.

Ryals, P. E. 2002. Smith-Somerville, H. S., and Buhse, H. E., Jr. 2002. Phenotypic switching in polymorphic *Tetrahymena*: a single-cell Jekel and Hyde. Int, Rev.Cytol., 242: 209-23.

Smith-Somerville, H. E., Hardman, J. K., Timkovich, R., Ray, W. J., Rose, K. E., Ryals, P. E., Gibbons, S. H., and Buhse, H. E., Jr. 2000. A complex of iron and nucleic acid catabolites is a signal that triggers differentiation in a fresh water protozoan. Proc. Nat. Acad. Sci. USA, 97: 7325-7330.

Ueno, K., Toshiaki, I., and Cheng, K. L. 1992. Handbook of Chemical Reagents. CRC Press, LLC Boca Ratan, Florida. p 14-15.

Table 1. The percentage of macrostomal differentiation induced by synthetically derived stomatin and compared to stomatin control.

Chelate Conc. (µg/ml)	Trial 1 % Differentiation	N	σ_X	Trial 2 % Differentiation	N	σ_X	Trials 1 & 2 % Differentiation	σ_x	Control (Stomatin) % Differentiation
1	11.8	5	7.9	4.4	5	0.8	8.2	6.8	9.8
5	7.5	5	5.4	5.4	5	1.2	6.4	4.1	7.7
10	6.1	5	1.7	5.1	5	1.2	5.6	1.6	6.7
50	51.1	5	7.4	45.2	5	7.1	48.1	7.8	57.7
		4							
100	68.7	3	9.5	58.6	5	6.4	65.3	8.9	78.4
Stomatin*	83.5	5	2.3	83.3	4	4.8	83.4	3.9	100
I.M.	6.9	5	6.9	5.9	5	2.1	6.4	2.1	7.6

*Stomatin 3mg/ml

Fig. 1. SEM of the oral apparatus of a microstomal cell showing one of the membranelles (M) and the undulating membrane (UM). The oral apparatus functions by filter feeding on bacteria and/or small particles. A current of water containing the bacteria and produced by the membranelles is forced through the undulating membrane which acts as a baleen trapping bacteria and particles which then fall into the cytostomal region and induce food vacuole formation.

Fig. 2. SEM showing the oral apparatus of a macrostomal cell. This carnivorous cell feeds by engulfing small ciliates through its large cytostomal opening (CY). Engulfed prey organisms become trapped in the cytopharyngeal pouch (PP) which eventually forms a food vacuole were digestion occurs. Membranelles 2 and 3 (M2 and M3, respectively) are indicated on the micrograph. Unlike the microstomal cell, the membranelles of the macrostomal form appear to have no role in prey capture. The 5 um bar shown in Fig. 1 applies to Fig. 2 as well.

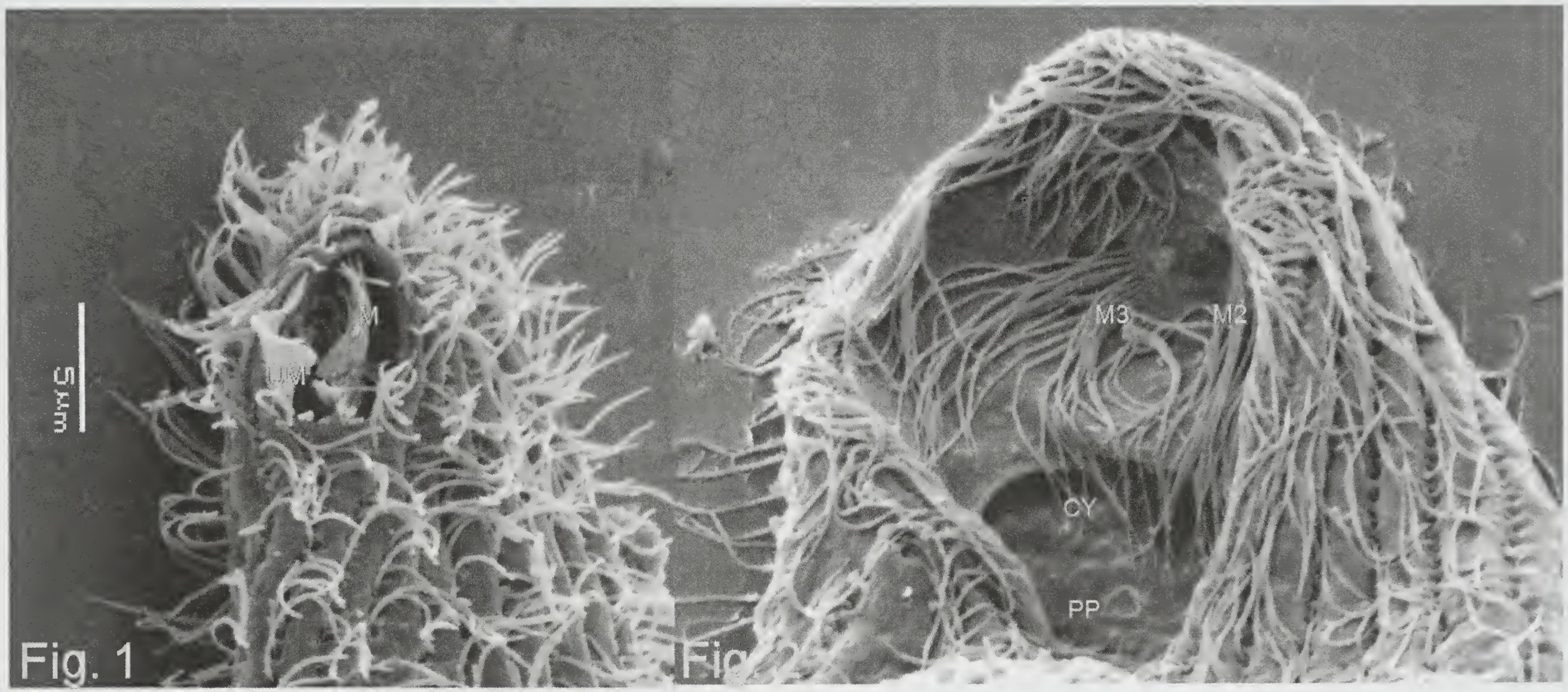


Fig. 1 Fig.

Fig. 3-6. *T. vorax* cells exposed to l ug/ ml chelate and 100 ug/ml synthetic chelate. The levels of macrostomal cells induced by inorganic medium (Fig. 3) were very low and equivalent to that induced by 1 ug/ml chelate (Fig. 4). The levels of differentiation initiated by 3 mg/ ml stomatin (Fig. 5) and 100 ug/ml chelate (Fig. 6) were similar, though the level of differentiation for chelate-treated cells were slightly lower (See Table I for a quantitative assessment). The brownish fiberous material visible in this photograph is most probably normal protein constituents of stomatin that are precipitated by Bouins fluid.

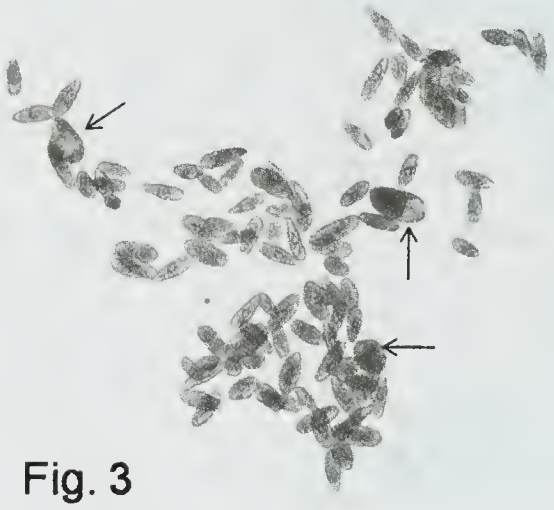


Fig. 3

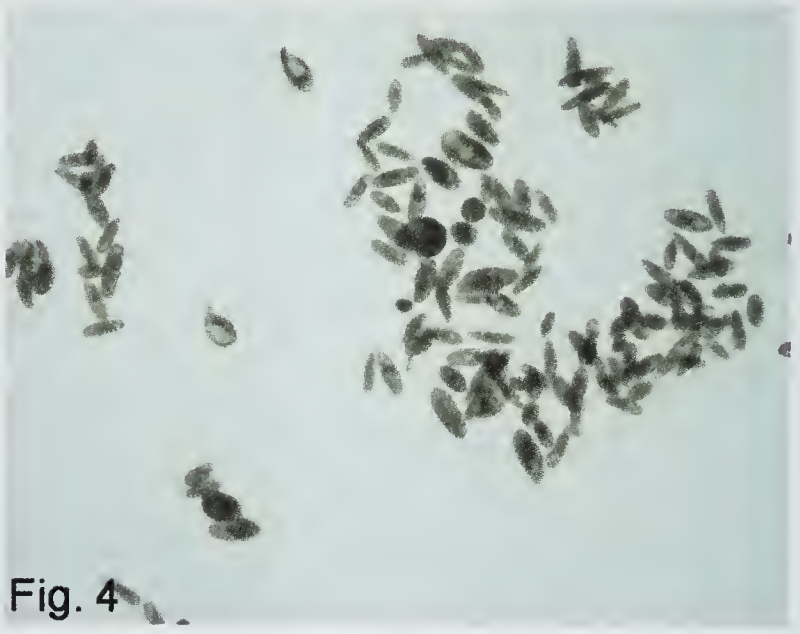


Fig. 4

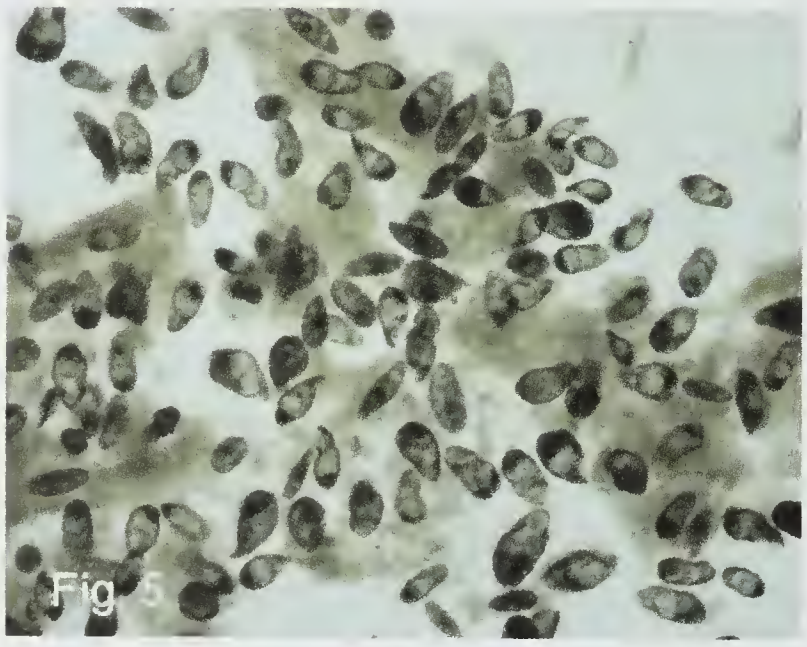
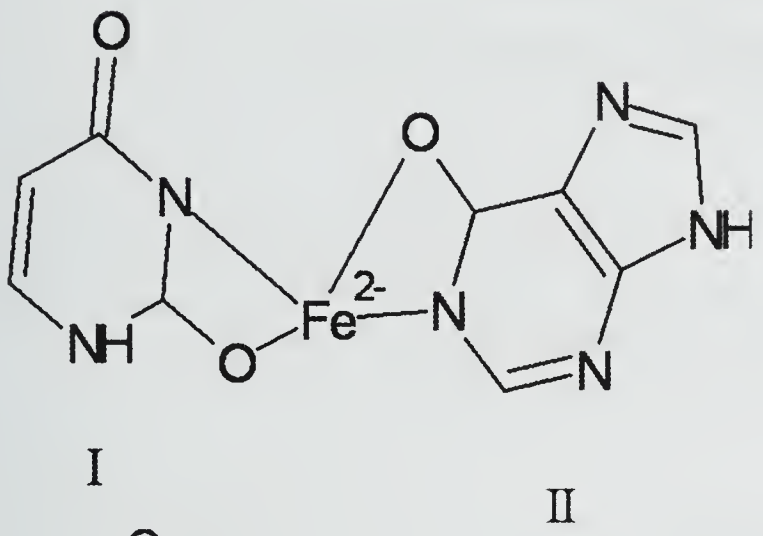
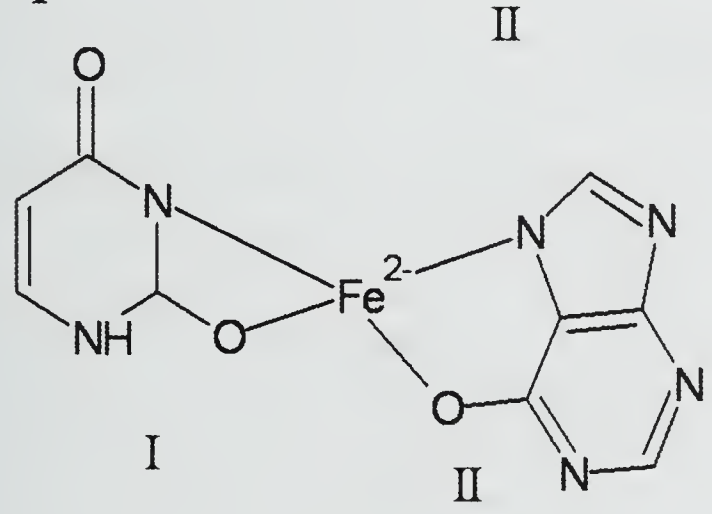


Fig. 5

Fig. 6

Fig. 7. Possible structures of the ferrous iron chelate based on coordination potential between iron hypoxanthine and uracil. However, many other structures are possible. Please see the text for more details.





I = Uracil

II = Hypoxanthine

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N:P Ratios and Temperature Affect Phosphate Uptake Rate in Phytoplankton Assemblages of a Cooling Water Reservoir

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ABSTRACT

Phytoplankton affinities for substrate uptake are influenced by temperature as well as nutrient concentration and may vary in response to environmental gradients. Newton Lake provides the opportunity to evaluate uptake rates by naturally occurring phytoplankton assemblages as influenced by heated effluent from a coal-fired power plant. We evaluated phosphate (PO_4-P) assimilation by assemblages from warm arm, forebay, and cold arm sites, which represent locations along a thermal gradient. Samples were collected and returned to the laboratory for *in vitro* determination of PO_4-P uptake at ambient temperature in response to nutrient additions. Treatment N:P ratios of 2.5:1 to 17.1:1 were intended to range from nitrogen to phosphorus limiting conditions. Under ambient conditions, PO_4-P uptake rates did not differ significantly among phytoplankton assemblages. When phosphate was limiting, uptake rates by warm arm and forebay assemblages were similar, but greater than that of the cold arm. At exceedingly high PO_4-P concentrations, any patterns of site-specific differences in uptake were not discernable.

INTRODUCTION

More than 80% of the natural landscape in Illinois has been converted to farmland (Rhoads and Herricks, 1996). With this change in landuse, a large proportion of waterways have been channelized, drained, and riparian zones removed in order to decrease flooding while increasing the amount of arable land for row crop agriculture (Infante et al., 2006; Talmage et al., 2002). Best management practices (BMPs) such as contour plowing, reduced tillage, buffer strips, and grassed waterways are very important to preserving stream integrity, but when not employed can increase the severity of agricultural disturbance (Allan and Castillo, 2007; Royer et al., 2006; Meador and Goldstein, 2003; Rabeni and Jacobson, 1999).

Impacts of agricultural practices also affect downstream bodies of water such as reservoirs. In agriculturally impacted regions, large quantities of sediment and nutrients flow into the reservoir. Watershed derived sediment and nutrients entering the reservoir can

create varying abiotic conditions along the longitudinal gradient (Wetzel, 2001). Therefore, phytoplankton assemblages may respond directly to such environmental variability.

In conjunction with increased sediment and nutrient inputs, rising water temperatures are of concern in agricultural watersheds due to removal of the riparian zones and soil compaction (Allan and Castillo, 2007; Sheehan and Rasmussen, 1999). Increased temperature can have profound impacts on aquatic communities which are adapted to narrow temperature ranges. Also, at high temperatures, the solubility of dissolved oxygen in water decreases resulting in hypoxic conditions. These increases in temperature and hypoxia may stress and ultimately extirpate aquatic communities (Herbert and Steffensen, 2005; Larson and Larson, 1996). Subsequently, eutrophication and algal blooms caused by nutrient loading may further exacerbate these conditions (Morgan et al., 2006; Royer et al., 2006).

Phytoplankton taxa exhibit various affinities for the uptake of a limiting substrate at different temperatures, and assemblage structure among thermally diverse habitats may differ along a spatial gradient (Raven and Geider, 1988). Numerous experiments have been conducted to determine the effect of temperature on unialgal cultures. For example, Rhee and Gotham (1988) found that above 15°C *Scenedesmus* sp. exhibits an increase in substrate uptake rate with a decrease in temperature. Conversely, several studies have documented an increase in nutrient uptake with an increase in temperature (Lim et al., 2006; Reay et al,. 2001; Viner, 1984; Eppley, 1972). In marine phytoplankton, Goldman (1977) found that substrate uptake rates varied widely with regard to taxon. Though laboratory conditions of unialgal cultures may not mirror natural conditions of a reservoir, it is important to consider temperature as a major influence on the differentiation of zonal phytoplankton assemblages.

Monitoring and management of a reservoir may require understanding of the manner in which thermal variation affects trophic states and seasonal cycles. To our knowledge, little research has been conducted to determine whether phytoplankton assemblages vary in their uptake of nutrients across a temperature gradient within a reservoir. Newton Lake, a power plant reservoir in east-central Illinois, provides the opportunity to test the nutrient kinetics of naturally occurring phytoplankton assemblages across a thermal gradient. Therefore, the objectives of this project were to 1) determine which nutrient was in limitation across the thermal gradient and 2) determine if the natural phytoplankton assemblages at three points along the gradient differed with respect to uptake rate of the limiting nutrient.

Because temperature is known to increase rates of reaction, our hypothesis was that uptake rate of the limiting nutrient would increase with increasing temperature. Furthermore, Elliot et al. (2006) showed that with an increase in temperature, algal growing season expanded and timing of seasonal blooms changed with regard to species. Therefore, relative to the distinct thermal zones of Newton Lake, we reasoned that there would be a lengthy growing season in the warmer areas of the reservoir relative to the cold area, and that species-specific spring and summer blooms will occur at different times in each of these areas. Altered normal seasonal succession of taxa may cause variation in uptake rate among sites along the temperature gradient. That is, due to thermal disturbance,

community structure may vary among sites at given times; this may lead to assemblage-wide differences in uptake rate.

METHODS

Study Site

Newton Lake, located in Jasper County in eastern Illinois, is a reservoir with a surface area of 718 hectares and a maximum depth of 12 meters. A coal burning power plant utilizes this lake as a source for cooling water. Water is drawn from the eastern (cold) arm of the lake and discharged into the western (warm) arm of the lake (Ameren, 2008). Three sample collection locations were used for this experiment with one located in the warm arm, one in the cold arm, and one in the forebay (Figure 1).

Preliminary Nutrient Determinations

Prior to the experiment, carbon, nitrogen, and phosphorus levels were determined for each location in order to establish which nutrient most likely limited phytoplankton productivity. Water samples were collected and taken to the laboratory where analyses were performed using standard methods (APHA, 1995). Inorganic carbon was established from total alkalinity by titration. Ammonia, nitrate and nitrite, and total phosphate and soluble reactive phosphate were determined using the phenate method, cadmium reduction method, and the ascorbic acid method, respectively. Because total alkalinity exceeded 48 mg $CaCO_3$ L^{-1}, we excluded carbon as a potential limiting nutrient (Moyle, 1949). The Redfield ratio (Redfield, 1958) suggests that phosphorus limitation occurs at N:P ratios greater than 7:1, as was the case in Newton Lake.

Experimental Protocol

Water samples were collected from the photic zone at each of the three locations on the following day using an integrated sampler fitted with 80-μm mesh Nytex® on the inlet, so as to exclude zooplankton. Depth of the photic zone was determined as 2x Secchi depth. All water samples were placed into acid-washed 2-L cubitainers and transported back to the laboratory. Sample aliquots were dispensed into clear 60-mL borosilicate vials which are certified to be free of any contaminants.

Three replicates of each sample were treated with one of seven different PO_4-P spikes making a total of 42 vials per location. PO_4-P spikes were formulated based on the preliminary nutrient concentrations (Table 1). Vials were incubated in the laboratory under ambient light in 40-L aquaria maintained within ± 1.3 °C of the temperature from which the samples were collected.

For each location, 42 vials were divided into short and long incubation time treatments of 21 vials apiece; the former incubation periods ranging from 32 to 55 min., the latter ranging from 212 to 245 min. Each incubation time treatment was further subdivided into three replicates of seven PO_4-P treatments. Following the short and long incubation periods, soluble reactive phosphorus in each sample was determined after passage through 0.45-µm pore nitrocellulose syringe filters to remove phytoplankton. For each location, rate of disappearance of PO_4-P was used as a surrogate for average uptake velocity (v) as mg PO_4-P mg^{-1} ash-free dry weight hr^{-1} and was calculated using the following formula:

$$v = \frac{\sum_{i=1}^{3}\left[\sum_{j=1}^{3}\left(ss_i - s_j\right)\right]}{\sum_{i=1}^{3}\left[\sum_{j=1}^{3}\left(tt_i - t_j\right)\right]}\left(\frac{1}{VSS}\right)$$

where

ss = phosphate concentration at end of incubation,
s = phosphate concentration at beginning of incubation,
tt = time at end of incubation,
t = time at beginning of incubation, and
VSS = volatile suspended solids.

Statistical Analyses

All data were analyzed utilizing SAS 9.1 software. An analysis of variance (ANOVA) was used to determine if there were significant differences among uptake rates across locations for each concentration. Duncan post hoc tests were performed to determine where differences occurred. Significance was determined as $P \leq 0.05$.

RESULTS

An array of descriptive physiochemical data were collected at all three study locations the day of the experiment (Table 1). Secchi depths for the warm arm, cold arm, and forebay were 27.45, 25.35, and 26.85, respectively. Cursory examination revealed that phytoplankton assemblages were similar at all three sample locations.

Treatments had a unique influence on phytoplankton assemblages at each sample location (Figure 2, Table 3). Under ambient conditions, PO_4-P uptake rates were indistinguishable among phytoplankton assemblages at all three locations. Though significant differences in uptake rate were not apparent between locations in treatment 2, the phytoplankton assemblage in the cold arm appeared to exhibit a lower uptake rate than warm arm or forebay assemblages. For treatments 2 and 3, as relative phosphorus availability increased, uptake rate of the cold arm assemblage decreased. Additionally, cold arm assemblage uptake rate was lower than that observed in the other two assemblages, though the difference was only significant for treatment 3. Uptake rates for treatments 4 and 5, those with moderate N:P ratios ranging from 6.6:1 to 9.1:1, were not significantly different among the assemblages. Treatments 6 and 7, which had very high relative phosphorus availability, produced no discernable pattern in uptake rate, although a significant difference was observed between cold and warm arm for treatment 6. For all treatments with significant differences in uptake rate, warm and cold arm grouped independently, whereas forebay samples were associated with either the warm or cold arm samples, depending upon treatment (Table 3).

DISCUSSION

Nutrient Limitation and the Redfield Ratio

Environmental stoichiometry aids in determining which nutrient may limit phytoplanktonic taxa. Redfield (1958) suggested that a ratio of 40C: 7N: 1P is useful for determining the nutrient which is in short supply relative to need. In experiments utilizing *Selenastrum capricornutum*, Chiaudani and Vighi (1974) demonstrated that phosphorus limitation occurred at N:P ratios in excess of 10:1, and that nitrogen limitation resulted when ratios were below 5:1. At N:P ratios between 5-10:1, they demonstrated that either N or P could be limiting depending upon other environmental factors. Our treatments were formulated to approximate this general categorical division of nutrient limitation.

Uptake Under P-limitation (Treatments 2, 3)

Uptake of PO_4-P by phytoplankton was found to be a function of temperature and concentration. Because no differences in PO_4-P uptake were found among the three assemblages under ambient conditions, differences in uptake rate in experimental treatments could be attributed to phosphate augmentation. Adaptation of phytoplankton to ambient conditions may explain the apparent decreased uptake rate by cold arm assemblages in treatments 2 and 3. Forebay and warm arm assemblages likely exhibited a real increase in uptake rate, whereas uptake rate of the cold arm assemblage was perceived to have decreased. This may have been an artifact of our methodology. According to Nalewajko and Lean (1980), measurement of uptake as PO_4- P remaining in the filtrate rather than the particulate fraction may cause a significant departure from the Michaelis-Menten equation. In actuality, cold arm assemblage uptake rate likely remained constant, but appeared to decrease since it was calculated as a function of PO_4-P availability. Likewise, uptake by forebay and warm arm assemblages may have appeared constant over a range of concentrations if assemblages were able to increase uptake rate to match PO_4-P availability.

Though temperature of the forebay (13.28°C) and cold arm (12.00°C) relate more closely with one another than with the warm arm (21.80°C), the forebay assemblage functions in a manner similar to that of the warm arm assemblage. Suttle and Harrison (1988) showed that *Scenedesmus* sp. under N-limitation and *Asterionella formosa* under PO_4-P limitation exhibit optimal uptake rates at 15°C and 19°-20°C, respectively. In the same sense, distinct assemblages also may have optimum temperatures for nutrient uptake as determined by their constituent species (Reay et al., 2001). Uptake rates of PO_4-P for assemblages in the warm arm and forebay could be similar in the event that ambient temperatures fall on either side of the optimum.

Uptake in Absence of P Limitation (Treatments 4, 5, 6, 7)

Nutrient additions in treatments 4 through 7 produced unrealistically high phosphate concentrations resulting in fluctuations in PO_4-P uptake rates that are not easily interpretable. Fuhs et al. (1972) and Spijkerman and Coesel (1998) suggested that such concentrations can cause deviations from standard uptake models and are unpredictable. Some phytoplankton occurring at low N:P ratios may modify their nutrient uptake and thereby alter environmental stoichiometry (Ketchum, 1939). Phytoplankton not only absorb PO_4-P, but also continuously exchange it with the extracellular environment (Kuenzler and Ketchum, 1962). Moreover, Ketchum (1939) demonstrated that uptake of PO_4-P was

dependent upon nitrate concentrations. Changes in the N:P ratio may result in either absorption or release of PO_4- P; thus, speculation on differences among treatments may be fruitless.

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LITERATURE CITED

Allan, D.J., and M.M. Castillo. 2007. Stream Ecology: Structure and Function of Running Waters, 2nd edition. Springer, Dordrecht, the Netherlands.

APHA, American Water Works Association, and Water Pollution Control Federation. 1995. A.E. Greenberg, A.D. Eaton, and L.S. Clesceri (eds.) Standard Methods for the Examination of Water and Wastewater, 19th edition. American Public Health Association, Washington, D.C.

Ameren. "Newton Power Plant". Ameren webpage. 2008. http://ameren.com/AEG/ADC_AU_Newton.asp

Chiaudani, G., and M. Vighi. 1974. The N:P ratio and tests with *Selenastrum* to predict eutrophication in lakes. Water Research 8: 1063-1069.

Elliot, J.A., I.D. Jones, and S.J. Thackeray. 2006. Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559: 401-411.

Eppley, R.W. 1972. Temperature and phytoplankton growth in the sea. Fishery Bulletin 70: 1063-1085.

Fuhs, G.S., E.M. Renger, E. Canelli, and M. Chiu. 1972. Characterization of phosphorus-limited plankton algae. American Society of Limnology and Oceanography Special Symposium 1: 113-133.

Goldman, J.C. 1977. Temperature effects on phytoplankton growth in continuous culture. Limnolology and Oceanography 22: 932-936.

Herbert, N.A., and J.F. Steffensen. 2005. The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. Marine Biology 147:1403-1412.

Infante, D.M., M.J. Wiley, and P.W. Seelbach. 2006. Relationships among channel shape, catchment characteristics, and fish in lower Michigan streams. American Fisheries Society Symposium 48: 339-357.

Ketchum, B.H. 1939. The absorption of phosphate and nitrate by illuminated cultures of *Nitzchia closterium*. American Journal of Botany 26: 399-407.

Kuenzler, E.J., and B.H. Ketchum. 1962. Rate of phosphorus uptake by *Phacodactylum tricornutum*. Biological Bulletin 123: 134-145.

Larson, L.L., and S.L. Larson. 1996. Riparian shade and stream temperature: a perspective. Rangelands 18: 149-152.

Lim, P., C. Leaw, and G. Usup. 2006. Effects of light and temperature on growth, nitrate uptake, and toxin production of two tropical dinoflagellates: *Alexandrium tamiyavaichii* and *Alexandrium minutum* (Dynophyceae). Journal of Phycology 42: 786-799.

Meador, M.R., and R.M. Goldstein. 2003. Assessing water quality at large geographic scales: relations among land use, water physiochemistry, riparian condition, and fish community structure. Environmental Management 31: 504-517.

Morgan, A.M., T.V. Royer, M.B. David, and L.E. Gentry. 2006. Relationships among nutrients, chlorophyll-α, and dissolved oxygen in agricultural streams in Illinois. Journal of Environmental Quality 35: 1110-1117.

Moyle, J.B. 1949. Some indices of lake productivity. Transactions of the American Fisheries Society 76: 322-334.

Nalewajko, C., and D.R.S. Lean. 1980. Phosphorus. Pages 235-258 *in* I. Morris (ed.) Studies in Ecology, Volume 7, The Physiological Ecology of Phytoplankton. University of California Press, Berkeley, California.

Rabeni, C.F., and R.B. Jacobson. 1999. Warmwater streams. Pages 505-525 *In*: C.C. Kohler and W.A. Hubert (eds.) Inland Fisheries Management in North America, 2nd edition, American Fisheries Society, Bethesda, Maryland.

Raven, J.A., and R.J. Geider. 1988. Temperature and algal growth. New Phytologist 110: 441-461.

Reay, D.S., J. Priddle, D.B. Nedwell, M.J. Whitehouse, J.C. Ellis-Evans, C. Deubert, and D.P. Connelly. 2001. Regulations by low temperature of phytoplankton growth and nutrient uptake in the Southern Ocean. Marine Ecology Progress Series 219: 51-64.

Redfield, A. 1958. The biological control of chemical factors in the environment. American Science 46: 205-221.

Rhee, G., and I.J. Gotham. 1988. The effect of environmental factors on phytoplankton growth: temperature and the interactions of temperature with nutrient limitation. Limnology and Oceanography 26: 635-648.

Rhoads, B.L., and E.E. Herricks. 1996. Naturalization of headwater streams in Illinois: challenges and possibilities. Pages 331-337 *in* A. Brooks and F.D. Sheilds Jr. (eds.) River Channel Restoration: Guiding Principles for Sustainable Projects. John Wiley and Sons, New York.

Royer, T.V., M.B. David, and L.E. Gentry. 2006. Timing of riverine export of nitrate and phosphorous from agricultural watersheds in Illinois. Environmental Science Technology 40: 4126-4131.

Sheehan, R.J., and J.L. Rasmussen. 1999. Large rivers. Pages 529-557 *in* C. C. Kohler and W. A. Hubert (eds.) Inland fisheries management in North America, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Spijkerman, E., and P.F.M. Coesel. 1998. Different response mechanisms of two planktonic desmid species (Chlorophyceae) to a single saturating addition of phosphate. Journal of Phycology 34: 438-445.

Suttle, C.A., and P.J. Harrison. 1988. Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. Limnology and Oceanography. 33: 186-202.

Talmage, P.J., J.A. Perry, and R.M. Goldstein. 2002. Relation of instream habitat and physical conditions of agricultural streams in the northern Midwest. North American Journal of Fisheries Management 22: 825-833.

Viner, A.B. 1984. Laboratory experiments on the effects of light and temperature on the uptake of nutrients by Lake Rotongaio phytoplankton. New Zealand Journal of Marine and Freshwater Research 18: 323-340.

Wetzel, R.G. 2001. Limnology: Lake and Reservoir Ecosystems, 3rd edition. Academic Press, New York.

Table 1. Physiochemical descriptors of Newton Lake locations collected on the day of sampling.

Variate	Warm Arm	Forebay	Cold Arm
Temperature (°C)	21.80	13.28	12.00
Specific Conductance (mS)	277	263	243
pH	7.40	7.09	7.25
Dissolved O_2 (mg O_2 L^{-1})	14.00	9.61	14.40
Total Phosphorus (mg PO_4-P L^{-1})	0.178	0.194	0.189
Soluble Reactive Phosphorus (mg PO_4-P L^{-1})	0.095	0.084	0.099
NO_2+NO_3 (mg L^{-1})	0.821	0.828	0.807
NH_4-N (mg L^{-1})	0.035	0.069	0.113

Table 2. Nitrogen availability relative to phosphorus for the warm, cold, and forebay sites of Newton Lake. Treatment 1 represents ambient relative to available nitrogen. Treatments 2-7 represent ambient plus phosphorus spike.

Treatment	Warm Arm	Cold Arm	Forebay
1	17.1	12.8	13
2	11.9	10.1	9.9
3	10.3	9	8.7
4	9.1	8.3	7.9
5	7.3	7	6.6
6	5	5	4.7
7	2.5	2.8	2.5

Table 3. ANOVA comparing influence of treatment on phytoplankton assemblage uptake rate for cold arm (C), warm arm (W), and forebay (F). Duncan post-hoc tests were performed on treatments with a significant $P \leq 0.05$.

Treatment	df	F value	*P*	Duncan grouping
1	2	1.6	0.2237	n/s
2	2	3.19	0.0593	n/s
3	2	31.27	<0.0001	W,F ≠ C
4	2	4.41	0.0234	W ≠ F,C
5	2	0.21	0.8149	n/s
6	2	7.83	0.0024	W,F ≠ C
7	2	0.72	0.4971	n/s
Error	22			

Figure 1. Map of Newton Lake in east-central Illinois, depicting three sampling locations. The warm arm (W), cold arm (C), and forebay (F) were sampled at N38.92444° W88.30254°, N38.91108° W88.29025°, and N38.89286° W88.30637°, respectively.

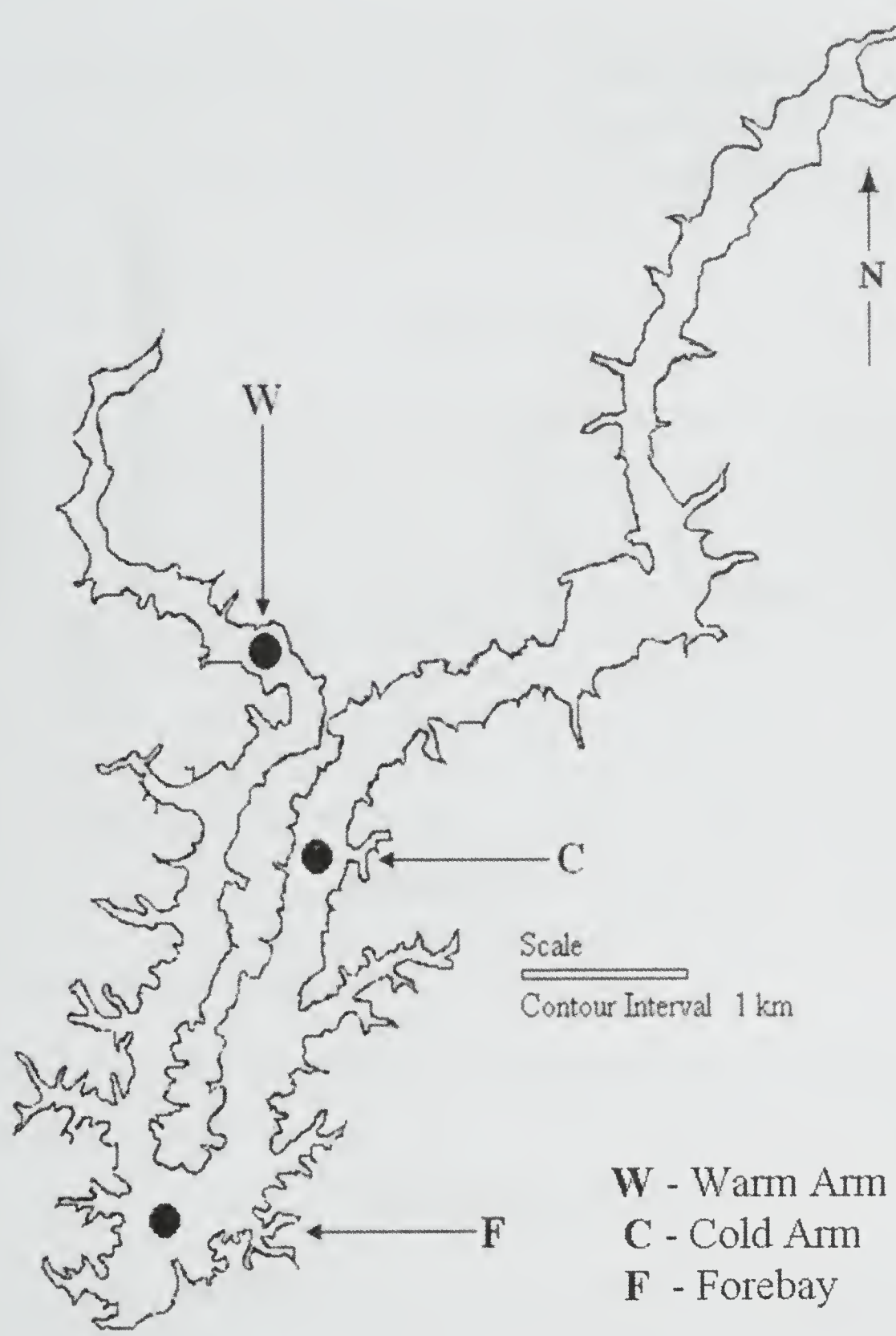
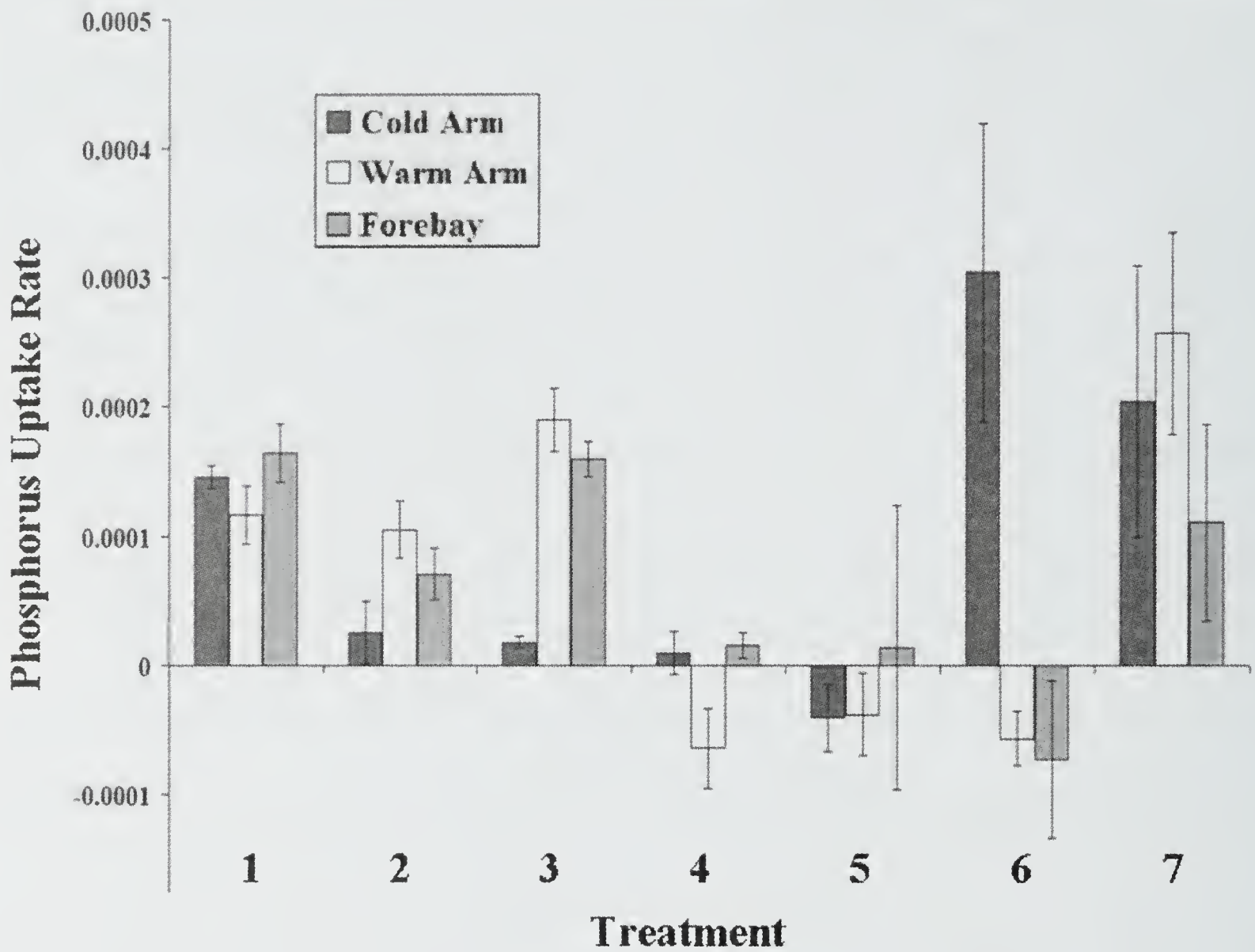


Figure 2. Mean uptake rate of phosphorus (mg PO_4-P mg VSS^{-1} hr^{-1}) for each treatment by location within Newton Lake (± standard error).



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Beaver Habitat Models for Use in Illinois Streams

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ABSTRACT

Although beavers are widely distributed in Illinois, their relative abundance varies among watersheds. We investigated the spatial distribution of beaver (*Castor canadensis*) colonies along the Embarras River in central Illinois, then tested two existing habitat models and developed a new logistic regression model to better assess the habitat requirements of this species. We located and mapped 125 colonies on the river, a mean of 0.40 colonies/km. Colonies tended to be uniformly distributed with a disproportionate number occurring one km apart. Of two habitat models tested, scores developed using the Habitat Suitability Index (HSI) model did not correlate with colony density ($r^2 = 0.111$; $P = 0.588$), but scores developed using a Missouri model did correlate with density ($r^2 = 0.578$; $P = 0.002$). Our logistic regression model ($r^2 = 0.584$, $P = 0.014$) indicated that the presence of beavers was positively correlated with sapling abundance near the river and width of the riparian zone, but negatively correlated with river gradient and road density. Intraspecific competition and changing environment appeared to be the primary forces influencing the spatial distribution of beavers along the river.

Key words: beaver, *Castor canadensis*, habitat models, logistic regression, HSI model

INTRODUCTION

The beaver (*Castor canadensis*) is an ecologically important keystone species capable of altering plant communities and creating vital wetlands through herbivory and water impoundment (Broschart et. al., 1989). Beavers are valued for their fur, but can be costly nuisances when their foraging damages valuable trees and dams cause flooding of roads and crops. Beaver populations have increased dramatically in Illinois during the past 30 years, but they are not distributed uniformly across the state and abundance varies regionally (Hoffmeister, 1989). Previous research showed that aerial surveys can be useful tools for comparing the relative abundance of this species in some watersheds (Woolf et al., 2003). However, these surveys can be problematic in stream habitats (which account for ~84% of all beaver habitat in southern Illinois) because fluctuating water level and tree canopy often obscure bank dens and food caches reducing the detectability of beavers (Woolf et al., 2003).

We investigated whether beaver-habitat models could be useful alternatives for predicting habitat quality and relative abundance of beavers in Illinois' streams. Our objectives were to: (1) map and quantify the distribution of beaver colonies, (2) test the efficacy of two existing models for predicting relative abundance, the Habitat Suitability Index (HSI) model and a Missouri habitat model, and (3) develop a new logistic regression model to predict relative abundance of colonies in Illinois' rivers.

METHODS

Study area

This study was conducted on the Embarras River in east-central Illinois, one of 9 major watersheds in Illinois. The river is typical of many Illinois rivers, is moderate-sized with a low gradient, and drains a large, flat watershed heavily impacted by agriculture. Water levels can fluctuate dramatically in part because the agricultural drainage systems move water quickly from crop fields. Over 50% of the river is classified as "biologically significant", a designation reserved for Illinois' highest quality streams (Wiggers, 1998).

Measuring habitat variables

Locations of all beaver colonies were mapped during November 2001 - February 2002 when bank dens, food caches, and chewed trees were most evident. The entire river was searched thoroughly during this period by canoe and on foot. Most colonies were identifiable based on the presence of dens in close proximity to food caches. When dens were not visible, a colony was defined as a stream segment >300 m in length with fresh sign (Robel et al., 1993). The location of each active colony was recorded in Universal Transverse Mercator (UTM) coordinates using a global positioning system (GPS) and marked on a 7.5-minute United States Geological Survey (USGS) topographic map.

Variables that could influence the quantity and quality of beaver habitat were selected *a priori* based on natural history and previous habitat models. These were measured in 26 2.5-km segments of river selected using a stratified-random scheme. First, the river was divided into upper, middle, and lower divisions, then each division was divided into 25-km sections, and two 2.5-km segments within each section were randomly selected for sampling. The UTM coordinates delineating the beginning and end of each segment were recorded from USGS maps and these were located in the field using GPS. In each segment, the number of colonies (dependent variable) and set of habitat variables (independent variables) were quantified.

Between June and August, we sampled vegetation in each river segment using five 100-m transects located perpendicular to the river at 500-m intervals on alternating banks. Sample points were established along each transect at 10-m intervals and species composition, diameter, and canopy cover of trees were estimated using the point-quarter technique and a densiometer. Shrub cover and height were estimated using the line-intercept method and a height pole (Cox, 2002). We measured channel width, bank height, and composition (silt, sand, or clay) at each transect. Width of the riparian zone, presence of agriculture fields, and number of roads within 200 m of the river, were measured on georectified aerial photographs. Stream gradients were extracted from the Illinois Stream Identification System (ISIS) database developed by the Illinois Department of Natural Resources (IDNR).

Testing habitat models

We first tested the U.S. Fish and Wildlife Service's habitat suitability index (HSI riverine habitat model) for beavers (Allen, 1983). Variables used in the model include: stream gradient, average water fluctuation (m), % canopy closure, % trees in the 2.5-15.2 cm diameter at breast height (dbh) class, % shrub cover, shrub height, and woody species composition within 200 m of the stream. We calculated the HSI score for each of 26 segments using the mean of transect data. Average water fluctuation was estimated based on minimum and maximum flow rates reported by the USEPA at gauging stations in Camargo, Ste. Marie, and Lawrenceville, IL. We used simple linear regression to test the relationship between HSI habitat scores and the number of colonies in each segment to test the efficacy of the HSI model for predicting relative abundance of beavers.

Next, we tested a model developed to quantify habitat suitability in the bottomland forests of Missouri (the Missouri model; Hallett and Erickson, 1980). To our knowledge, this is the only model designed specifically for use in the riverine habitats of the Midwest. However, its validity had not been tested prior to our study. Variables used in the model include: bank texture and slope, tree species composition and dbh, number of important food plants, proximity of crop fields, and presence of permanent water. The model provides the user with the option of removing habitat characteristics not applicable to a site. Because all of the river segments provided permanent water, we deleted the latter variable from the model and adjusted scores accordingly (Hallett and Erickson, 1980). Again, the relationships between habitat scores and the number of colonies in each segment were tested using linear regression.

Finally, we developed a new beaver habitat model using forward logistic regression to determine which of 12 independent habitat variables (Table 1) could be used to predict the presence/absence of beavers in each 2.5-km stream segment. To avoid potential multi-collinearity between variables in the model, we first conducted Spearman correlation analyses and eliminated 3 variables that were highly correlated ($P < 0.05$) with other more easily measured variables. We set the threshold necessary for a variable to enter the model at ≤ 0.15 so as not to exclude any that might be biologically important to beavers. Each of the four variables included in the final model were accompanied by a significant ($P < 0.1$) change in the F-value associated with the overall regression. Spearman correlations and logistic regression analyses were performed using SPSS software (SPSS Inc. Chicago, IL).

RESULTS

Density and spatial distribution of colonies

We located and mapped 125 colonies on the Embarras, a mean of 0.40 colonies/ km (Figure 1). Based on nearest-neighbor distances, colonies tended to be uniformly distributed along the river, with a disproportionate number occurring approximately 1-km apart ($X^2 = 32.6$; $P < 0.01$; Figure 2). The minimum distance between adjacent colonies was 400 m.

Twenty of the 26 (76.9%) river segments contained active beaver colonies; only six segments lacked colonies. Of the segments with colonies, nine had a single colony and 11

contained two colonies. The majority (97.6%) of colonies occupied bank dens; only three occupied lodges and these were all located in the headwaters of the river. Similarly, only two dams were found on the main channel of the river, both in the upper reaches where the river was narrow and flow was low.

Of the habitat characteristics measured, only stream gradient, correlated significantly with colony density ($r = -0.440$, $P = 0.024$). The gradient was lowest in the upper reaches and near the river mouth where densities tended to be high. In contrast, middle sections of the river had the highest gradient and colonies were sparse. Several other habitat parameters approached statistical significance, including percentage of the river with low banks ($r = 0.363$, $P = 0.068$), riparian width ($r = 0.355$, $P = 0.075$), shrub cover ($r = 0.351$, $P = 0.079$), and canopy cover ($r = 0.337$, $P = 0.092$).

Testing existing habitat models

HSI scores ranged from 0.0 to 1.0, with a mean = 0.82 (SD = 0.28), suggesting that the quality of beaver habitat varied considerably along the river, but generally was good. The only segment with unsuitable habitat (HSI = 0.0) had no beavers present and the segments with highest colony densities had optimal habitat according to the model. However, overall HSI scores did not correlate well with the number of colonies ($r^2 = 0.111$, $P = 0.588$). For example, four segments with excellent habitat (HSI > 0.8) had no colonies, whereas six segments with only moderate habitat (HSI ~ 0.5) contained high densities.

Habitat scores derived from the Missouri model correlated significantly with colony density ($r^2 = 0.578$; $P = 0.002$). Scores ranged from 43% to 71% (mean = 59%, SD = 6.7), again suggesting habitat along much of the river was good. Segments with highest scores also had the greatest number of colonies. Segments lacking colonies earned scores ranging from 43% to 60%. Segments with lowest scores lacked a forested riparian zone and provided little winter food after crops were harvested. Variables that most influenced habitat scores on the Embarras River were size class of trees and bank texture. Segments dominated by large, mature trees or with sandy banks unsuitable for dens received low scores.

Logistic regression model

We developed a regression model that retained four independent variables: % riparian trees <15 cm dbh, riparian zone width, stream gradient, and number of roads within 200 m. The resulting standardized regression coefficients (Tree < 15 B= 0.075, RZW B = 0.030, Grad B = -0.760, Roads B = -0.723, and a constant of -0.642) indicated the relative importance of each variable to the model. Probability of a stream segment being occupied by beavers increased with relative abundance of small trees and wide riparian zones and decreased with stream gradient and road density. The resulting model was a significant predictor of the presence/absence of beavers ($r^2 = 0.58$, $P = 0.014$) and successfully predicted their presence/absence in 24 of 26 (92%) stream segments, including all 20 where beavers were present and four of six segments where beavers were apparently absent.

DISCUSSION

Density and spatial distribution of colonies

With a mean of 0.40 colonies/km of stream, the Embarras River provides good quality beaver habitat along most of its length. Robel et al. (1993) found that rivers with good beaver habitat had densities of 0.12 to 1.40 colonies/km in Kansas and Semyonoff (1951 *in* Novak, 1987) found mean densities of 1.5 colonies/km for rivers with good habitat, 0.5 colonies/km in moderate habitat, and 0.1-0.2 colonies/km in poor habitat.

Distribution of colonies varied along the length of the watershed, reflecting changing environments along the river. Colony density was highest in the headwaters characterized by slow moving water, a narrow channel, and a broad floodplain that provided beavers with the opportunity to build lodges, dams, and bank dens. Topography is very flat and prone to flooding; consequently, farmers have removed some low areas from crop production and these ephemeral wetlands provide refuge and habitat during periods of flooding and drought. As flows and channel width increase downriver, beavers build fewer dams and are less capable of altering their local environment to create preferred habitat.

The Embarras watershed, like much of central Illinois, is dominated by corn and soybean fields. Robel et al. (1993) found that beavers in Kansas were as likely to forage on corn and sorghum as preferred trees such as cottonwood and willows. Beavers in our study area fed on corn and soybeans in the fall and corn stalks were evident in many food caches. However, after crop harvests, the landscape changes and the use of woody vegetation by beavers increased. Consequently, during winter, availability, composition, and stem size of woody plants likely influences habitat quality (Boyce, 1981). We found beavers to be most abundant in stream segments where periodic flooding maintained early-successional riparian forests dominated by small diameter trees.

Generally, as bank height and channel volume increased in lower portions of the watershed, the proportion of large trees (>45 cm dbh) increased and woody understory decreased, as did beaver density. Beavers inhabiting the lower portion of the Embarras River have adapted to fluctuating water levels. Trails from the river into cornfields and foraging areas frequently extended up steep banks and den openings were stacked vertically allowing use of different den openings depending on water levels.

Beavers are highly territorial and social interactions should lead to a uniform dispersion in suitable habitat assuming resources are evenly distributed (Davies, 1978). Uniform spacing of colonies along the Embarras River, particularly in the middle and lower portions suggests that territoriality, rather than resource limitation, is an important factor influencing distribution. A greater proportion of colonies occurred approximately one km apart than would be expected by chance. This is consistent with reported home ranges of approximately 0.8 km on streams (Nordstrom, 1972). Busher (1983) reported intercolony distances ranged from 0.84-1.55 km in California streams.

Testing existing habitat models

Habitat models have proven to be useful tools for quantifying habitat quality and relative abundance of beavers in streams and wetlands throughout North America (Slough and

Sadlier, 1977; Allen, 1983; Howard and Larson, 1985; Broschart et al., 1989). We tested Allen's (1983) HSI model because it is widely used for environmental impact assessments throughout the U.S. The model was not developed specifically to predict beaver densities, Robel et al. (1993) noted that HSI scores should be positively correlated with beaver densities if the model is composed of key habitat variables. Robel et al. (1993) and Stromayer (1999) reported poor performance for the HSI model in the midwestern and eastern U.S., respectively. Model limitations in these regions included its failure to incorporate local plant species as high quality foods and narrow definitions of suitable water quality and stream substrates.

The HSI model did not produce useful estimates of beaver density on the Embarras River and probably is not useful for estimating relative abundance of beavers in Illinois' watersheds. Correlations between HSI scores and colony densities were low ($r^2 = 0.111$; $P = 0.588$) in part because the model is based on characteristics more typical of beaver habitat in the northern and western portions of the geographic range, emphasizing winter foods and stream characteristics not typical of Illinois. For example, the model fails to incorporate regional foods such as corn, maple, and ash, and it defines suitable stream characteristics too narrowly, particularly water levels and substrates (Robel et al., 1993; Stromayer, 1999). On the Embarras River, beavers have adapted to fluctuating water levels and steep banks, as long as water depth is sufficient for travel and protection.

In contrast, the Missouri model proved well-suited for predicting the quality of beaver habitat in Illinois. Model variables such as bank characteristics, forest age and composition, important food plants, and distance to cropland are appropriate descriptors of beaver habitat in Illinois. The model captured the importance of tree species, size, and regional food plants (including crops), to beavers in the Embarras River watershed. Our results suggest that the Missouri model could be a useful tool for evaluating the quality of beaver habitat in Illinois and advances in remote sensing and GIS systems will allow future refinement of statewide habitat maps.

Logistic regression model

Our final regression model was a significant predictor of beaver presence, retaining variables that we believe represent important habitat components of Illinois streams. Of 12 habitat characteristics entered into the regression, four were retained in the final model. Two (abundance of small trees and riparian zone width) were positively associated with beaver colonies and two (stream gradient and proximity of roads) were negatively associated.

Habitat models are most useful when they incorporate variables that are easily quantifiable and produce results that can be clearly interpreted (Hurley, 1984; Salwasser, 1984; Garshelis, 2000). Our regression model is useful because variables are habitat features important to beavers and can be derived from existing data sets (watershed surveys and aerial photographs) eliminating the need to measure them in the field.

Riparian trees provide important food for beavers during winter when herbaceous vegetation is dormant and crops have been harvested. Beavers forage on small diameter woody stems during winter and use these to construct dens and dams. We frequently observed willows (*Salix* spp,), maples (*Acer saccharinum*, *A. saccharum*), and green ash

(*Fraxinus pennsylvanicum*) in food caches and dams. Consequently, it is not surprising that availability of small trees and the extent of riparian zone were important factors associated with beaver presence. Mature riparian forests dominated by large trees appear less suitable for beavers, perhaps because felling large trees is labor-intensive, their shade reduces understory growth, and their presence suggests infrequent flooding and scouring, disturbances that favor the early-successional plants used by beavers.

Two variables (stream gradient and road proximity) were negatively associated with beaver colonies. It is not surprising that beavers avoid areas with high gradients. Typically, higher gradients result in a higher flow which makes travel and transportation of food more difficult, and destroys dams, dens, and food caches. We believe that road proximity provides an indirect measurement of human activity along the river. Although beavers often live in close proximity to humans, previous research suggests that roads, railways, and land development near waterways limit habitat quality (Slough and Sadleir, 1977).

In conclusion, our research suggests that the Embarras River provides good quality beaver habitat along much of its extent. Although the HSI model was not a useful predictor of relative abundance of beavers on the river, both the Missouri model and our regression model produced scores that were correlated with beaver abundance. We believe this river is typical of many in Illinois and therefore these habitat models are likely to be useful for evaluating habitat quality and relative abundance of beavers in similar watersheds throughout the state.

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LITERATURE CITED

Allen, A. W. 1983. Habitat suitability index models: beaver. U. S. Fish and Wildlife Service, FWS/OBS-82/10.30. 20 pp.

Boyce, M. S. 1981. Habitat ecology of an unexploited population of beavers in interior Alaska. M.S. Thesis, University of Alaska, Fairbanks.

Broschart, M. R., C. A. Johnston, and R. J. Naiman. 1989. Predicting beaver colony density in boreal landscapes. Journal of Wildlife Management 53:929-934.

Busher, P. E. 1983. Interactions between beavers in a montane population in California. Acta Zoologica Fennica 174:109-110.

Cox, G. W. 2002. General ecology laboratory manual. 8th ed. McGraw-Hill Co. Inc. New York, NY. 312 pp.

Davies, N. B. 1978. Ecological questions about territorial behaviour. Pages 317–350 *in* J. R. Krebs and N. B. Davies (eds.). Behavioural ecology: an evolutionary approach. Sinauer, Sunderland, Mass. 494 p.

Garshelis, D. L. 2000. Delusions in habitat evaluation: measuring use, selection, and importance. Pages 111-164 *in* L. Boitani and T. K. Fuller (eds.). Research techniques in animal ecology. Columbia University Press, New York, N.Y.

Hallett, D. L. and D. W. Erickson. 1980. Beaver (*Castor canadensis*). Pages 99-105 *in* T. S. Baskett, D. A. Darrow, D. L. Hallett, M. J. Armbruster, J. A. Ellis, B. F. Sparrowe, and P. A.

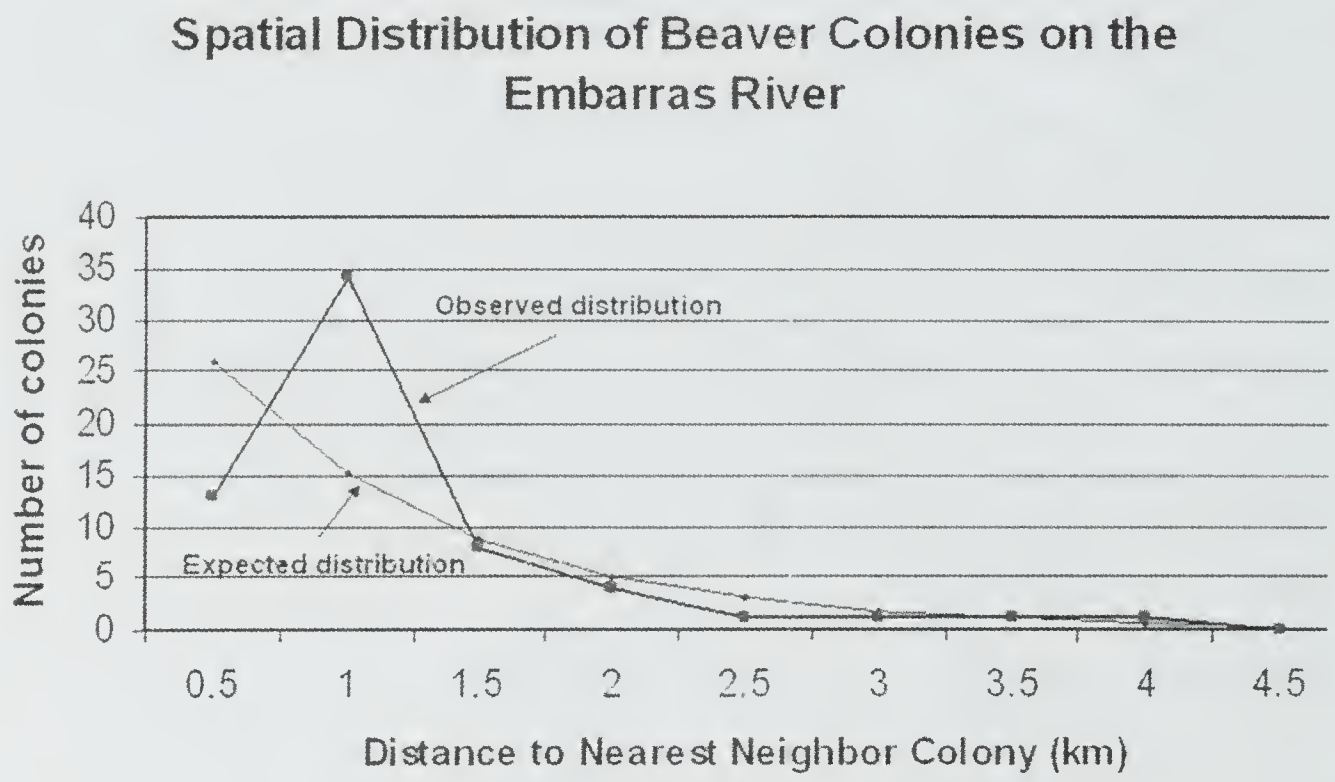
Korte (eds.) A handbook for terrestrial habitat evaluation in central Missouri. U. S. Department of Interior, Fish and Wildlife Service Resource Publication 133. Washington, D. C.
Hoffmeister, D. 1989. Mammals of Illinois. University of Illinois Press, Urbana and Chicago, IL. 348 pp.
Howard, R. J. and J. S. Larson. 1985. A stream habitat classification system for beaver. Journal of Wildlife Management 49:19-25.
Hurley, J. F. 1986. Development, testing, and application of wildlife-habitat models - the manager's viewpoint. Pages 151-153 *in* J. Verner, M. L. Morrison, and C. John Ralph (eds.). Wildlife 2000: modeling habitat relationships of terrestrial vertebrates. The University of Wisconsin Press, Madison, WI.
Nordstrom, W. R. 1972. Comparison of trapped and untrapped beaver populations in New Brunswick. M.S. thesis, University of New Brunswick, Fredricton. 104 pp.
Novak, M. 1987. Beaver. Pages 283-312 *in* Wild furbearer management and conservation in North America. M. Novak, J. A. Baker, M. E. Obbard and B. Malloch, (eds.) Ontario Trappers Association, North Bay, Ontario, Canada.
Robel, R. J., L. B. Fox, and K. E. Kemp. 1993. Relationship between habitat suitability index values and ground counts of beaver colonies in Kansas. Wildlife Society Bulletin 21:415-421.
Salwasser, H. 1986. Modeling habitat relationships of terrestrial vertebrates - the manager's viewpoint. Pages 419-424 *in* J. Verner, M. L. Morrison, and C. J. Ralph (eds.). Wildlife 2000: modeling habitat relationships of terrestrial vertebrates. The University of Wisconsin Press, Madison, WI.
Semyonoff, B. T. 1951. The river beaver in Archangel Province. Pages 5–45 in Russian game report. Vol. 1. Can. Dep. North. Aff. and Nat. Resour., Ottawa, Ont. (Transl. from Russian.)
Slough, B. G., and R. M. F. S. Sadlier. 1977. A land capability classification system for beaver (*Castor canadensis* Kuhl). Canadian Journal of Zoology 55:324-335.
Stromayer, K. A. K. 1999. Developing management alternatives to promote beaver occupancy. Abstract of paper presented at the Virginia Wildlife Society Meeting, Virginia Tech University, Blackburg, VA.
Wiggers, R. 1998. The Embarras River Basin: an inventory of the region's resources. A project of the critical trends assessment program, Illinois Department of Natural Resources, Springfield, IL.
Woolf, A., T. Nelson, and R. Bluett. 2003. Beaver relative abundance in southern Illinois watersheds. Transactions of the Illinois State Academy of Science 96:135-144.

Table 1. Twelve habitat variables tested for use in the final logistic regression model for beaver habitat in the Embarras River Watershed, central Illinois.

Variable	Units	Abbreviation
Canopy cover	%	CC
Riparian trees >45-cm dbh	%	Trees>45
Riparian trees <15-cm dbh	%	Trees<15
Shrub cover	%	SC
Shrub height	m	ShrubHt
Riparian zone width (mean)	m	RZW
Channel width (mean)	m	CW
Stream gradient	%	Grad
Bank height	m	BankHt
Stream sinuosity	%	Sinuous
No. roads within 200 m	--	Roads
No. cropfields within 200 m	--	Crops

Figure 1. Distribution of 125 beaver colonies observed along the Embarras River in eastern Illinois during November 2001 - February 2002. Each dot represents one colony.

Figure 2. Observed and expected distributions of nearest-neighbor distances between beaver colonies. The expected exponential distribution would occur if colonies were distributed randomly along a river. The dispersion of colonies was more uniform than would be expected by chance ($X^2 = 32.6$; $P < 0.01$).



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Thermoregulation of Male *Elaphe spiloides* in an Agriculturally-Fragmented Forest in Illinois

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ABSTRACT

Anthropogenic forest fragmentation increases the amount of edge habitat. Although edges are harsh environments for many native species, ratsnakes often prefer this habitat. We examined thermoregulatory effectiveness of Central Ratsnakes (*Elaphe spiloides*) using forest edges preferentially to determine if edge preference is driven by increased thermoregulatory efficiencies. Six male subjects were located every 1-2 days using radio-telemetry and temperature sensitive transmitters. Subjects did not thermoregulate more efficiently in edges than in forest. Snakes were thermoconformers in both habitat types suggesting edge preference might be driven by other factors.

Key Words: Central Ratsnake, *Elaphe spiloides*, Radio-telemetry, Thermoregulation

INTRODUCTION

Forest fragmentation resulting from agricultural practices is prevalent in the midwestern US, particularly Illinois (70% cropland; Bretthauer and Edgington 2003). In addition to a loss of forest, this practice is responsible for creating large amounts of edge habitat. Edges are subjected to more extreme abiotic influences (i.e., wind and temperature patterns, see Saunders et al. 1991), increased predation and/or competition among species (Bolger et al. 1991, Donovan et al. 1995, McCollin 1998), and proliferation of exotic species (Yahner 1988, Murcia 1995) making this habitat type harsh to many organisms. However, ratsnakes (*Elaphe* spp.) preferentially use edge habitat (Weatherhead and Charland 1985, Durner and Gates 1993, Blouin-Demers and Weatherhead 2002).

Edge preference of *Elaphe* might reflect an increased number of avian (Gates and Gysel 1978, Paton 1994) and small mammalian prey (Weatherhead and Charland 1985, Blouin-Demers and Weatherhead 2001a). Alternatively, because edges experience warmer temperatures (Flaspohler et al. 2001, Kolbe and Janzen 2002) due to increased sun exposure (Weatherhead and Charland 1985), edge preference might be due to increased ability to thermoregulate in this habitat. Thermoregulation facilitates quicker digestion of meals,

decreases gestation time of gravid females, and is critical during ecdysis. Therefore, thermoregulation might be the most important factor in determining squamate habitat use patterns (Grant 1990, Peterson et al. 1993, Reinert 1993). Ratsnakes in the northeastern US (*E. spiloides*, following Burbrink 2001) thermoregulate more efficiently in edges (Blouin-Demers and Weatherhead 2001a), but little is known about the thermoregulatory abilities of *Elaphe* in other geographic regions. We examine the thermoregulatory abilities of Central Ratsnakes (*Elaphe spiloides*) preferentially using edge habitat in Clark County, Illinois (see Foster et al. 2006), and test the hypothesis that this preference is due to increased thermoregulatory abilities in this habitat.

MATERIALS AND METHODS

This study was undertaken between 20 May 2003 and 7 November 2004 on ca. 280 ha of privately-owned land located 14 km southeast of Martinsville, Clark County, Illinois. Agriculture row-crops (soybeans [*Glycine max*] and corn [*Zea mays*]) surround and fragment a mixed mesic hardwood forest in a ridge-valley landscape, creating a large amount of edge habitat. Numerous creeks flow into a man-made lake of ca. 30 ha, which has further fragmented the forest habitat. A county road and power line bisecting the site provide additional edge habitat.

Radio transmitters with thermistors (calibrated prior to implantation; model SI-2T, Holohil Systems, Ltd., Ontario, Canada) were surgically implanted into collected ratsnakes (see Reinert 1992, Hardy and Greene 1999, 2000). Subject body temperature (± 0.1 °C) was calculated without disturbance to the snake based on the rate of emitted pulses. These values were compared to ambient temperatures recorded at the time of subject location. All subjects were located daily or on alternate days using a Telonics TR-4 receiver (Wildlife Materials, Inc., Carbondale, Illinois).

Because ratsnakes exhibited preference for edges over forest (Foster et al. 2006), thermoregulation data were analyzed in these habitats. The preferred body temperature range (T_{set}) was assumed 26.5-29.8 °C (following Blouin-Demers and Weatherhead 2001b). Following Blouin-Demers and Weatherhead (2002): $d_a - d_b$ = effectiveness of thermoregulation, where d_a is the deviation of ambient temperature from T_{set}, and d_b is the deviation of snake body temperature from T_{set}. We used this equation to compare thermoregulatory abilities between forest and forest edges (following Blouin-Demers and Weatherhead 2001c, defined as ± 15 m of the boundary between forest and any open habitat). Negative values of $d_a - d_b$ arise when snakes use thermally favorable habitats less than their availability, zero represents thermoconformity, and positive values indicate snakes that are thermoregulating efficiently. Values of d_a and d_b were not calculated for subjects in hibernation (between 5 November 2003 and 6 April 2004). Univariate analyses of variance (ANOVAs) were used to analyze thermoregulation data; non-independence of data was corrected by treating each snake as a block. All statistical analyses were calculated using SPSS (SPSS Inc., 2003).

RESULTS

Six male ratsnakes (mean snout-vent length ± 1 standard error = 119.8 ± 11.3 cm) were located 186 times in forest and 119 times in edges. Mean (± 1 SE) ambient temperature

and snake body temperature during locations was 23.6 ± 0.3 °C and 23.2 ± 0.3 °C, respectively. Ambient temperatures ranged from 5.2 to 36.3 °C and body temperature ranged from 9.5 to 35.7 °C. Ambient temperature exceeded body temperature 59.2% of all location events. Snake body temperatures were correlated with ambient temperatures for each individual ($r^2 \geq 0.37$, $p < 0.001$) and for data pooled among individuals ($r^2 = 0.73$, $p < 0.001$; Fig. 1).

Ambient temperatures and body temperatures did not differ between forest and edges ($F_{1,302} = 1.14$, $p = 0.29$ and $F_{1,302} = 0.46$, $p = 0.50$, respectively; Table 1). Snake body temperatures were within the preferred temperature range 18.8% of locations in forest habitat and 10.2% in edges, whereas ambient temperatures fell within this range 22% of the time in forest and 11.9% of the time in edges. Thermoregulatory effectiveness did not differ among subjects while occupying edge or forest habitat ($F_{5,4.462} = 3.47$, $p = 0.11$) or between edges and forest ($F_{1,4.611} = 0.40$, $p = 0.56$; Table 1).

DISCUSSION

Ambient temperatures at our study site were not higher in edges than forest, contrary to other studies (e.g., Flaspohler et al. 2001, Kolbe and Janzen 2002). Male ratsnake thermoregulatory effectiveness did not differ between these two habitats, indicating that edge preference (see Foster et al. 2006) might be due to other factors, possibly an increased number of prey (Gates and Gysel 1978, Weatherhead and Charland 1985, Blouin-Demers and Weatherhead 2001c).

Although we expected to find snakes maintaining body temperatures in the preferred thermal range (assumed 26.5-29.8 °C, following Blouin-Demers and Weatherhead 2001a), this was rarely the case. Several factors suggest that subjects were not attempting to achieve body temperatures in this range. Ambient temperatures were in this range more frequently than snake body temperatures in both habitats. Additionally, snake body temperatures varied greatly and were dependent upon ambient temperatures (Fig. 1). Calculated values for thermoregulatory effectiveness were close to zero in both forests and edges. Both are indicative of thermoconformity rather than active thermoregulation.

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LITERATURE CITED

Blouin-Demers, G., and P.J. Weatherhead. 2001a. An experimental test of the link between foraging, habitat selection and thermoregulation in black rat snakes *Elaphe obsoleta obsoleta*. Journal of Animal Ecology 70:1006-1013.

Blouin-Demers, G., and P.J. Weatherhead. 2001b. Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. Ecology 82:3025-3043.

Blouin-Demers, G., and P.J. Weatherhead. 2001c. Habitat use by black rat snakes (*Elaphe obsoleta obsoleta*) in fragmented forests. Ecology 82:2882-2896.

Blouin-Demers, G., and P.J. Weatherhead. 2002. Habitat-specific behavioral thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). Oikos 97:59-68.

Bolger, D.T., A.C. Alberts, and M.E. Soule. 1991. Occurrence patterns of bird species in habitat fragments: Sampling, extinction, and nested species subsets. The American Naturalist 137:155-166.

Bretthauer, S.M., and J.M. Edgington. 2003. Illinois Forests. Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois, USA.

Burbrink, F.T. 2001. Systematics of the eastern ratsnake complex (*Elaphe obsoleta*). Herpetological Monographs 15:1-53.

Crother, B.I., J. Boundy, J.A. Campbell, K. De Quieroz, D. Frost, D.M. Green, R. Highton, J.B. Iverson, R.W. McDiarmid, P.A. Meylan, T.W. Reeder, M.E. Seidel, J.W., Sites, Jr., S.G. Tilley, and D.B. Wake. 2003. Scientific and standard English names of amphibians and reptiles of North America north of Mexico: Update. Herpetological Review 34:196-203.

Donovan, T.M., R.H. Lamberson, A. Kimber, F.R. Thompson, III, and J. Faaborg. 1995. Modeling the effects of habitat fragmentation on source and sink demography of Neotropical migrant birds. Conservation Biology 9:1396-1407.

Durner, G.M., and J.E. Gates. 1993. Spatial ecology of black rat snakes on Remington Farms, Maryland. Journal of Wildlife Management 57:812-826.

Flaspohler, D.J., S.A. Temple, and R.N. Rosenfield. 2001. Effects of forest edges on ovenbird demography in a managed forest landscape. Conservation Biology 15:173-183.

Foster, C.D., S. Klueh, and S.J. Mullin. 2006. *Elaphe spiloides* (Central Ratsnake). Habitat Use. Herpetological Review 37:478.

Gates, J.E., and L.W. Gysel. 1978. Avian nest dispersion and fledgling success in field-forest ecotones. Ecology 59:871-883.

Grant, B.W. 1990. Trade-offs in activity time and physiolocial performance for thermoregulating desert lizards, *Sceloporus merriami*. Ecology 71:2323-2333.

Hardy, D.L., Sr., and H.W. Greene. 1999. Surgery on rattlesnakes in the field for implantation of transmitters. Sonoran Herpetology 12:25-27.

Hardy, D.L., Sr., and H.W. Greene. 2000. Inhalation anesthesia of rattlesnakes in the field for processing and transmitter implantation. Sonoran Herpetology 13:110-114.

Kolbe, J.J., and F.J. Janzen. 2002. Spatial and temporal dynamics of turtle nest predation: Edge effects. Oikos 99:538-544.

McCollin, D. 1998. Forest-edges and habitat selection in birds: A functional approach. Ecography 21:247-260.

Murcia, C. 1995. Edge effects in fragmented forests: Implications for conservation. Trends in Ecology and Evolution 10:58-62.

Paton, P.W. 1994. The effect of edge on avian nest success: How strong is the evidence? Conservation Biology 8:17-26.

Peterson, C.R., A.R. Gibson, and M.E. Dorcas. 1993. Snake thermal ecology: The causes and consequences of body-temperature variation. Pp. 241-314 *In* Snakes: Ecology and Behavior. Seigel, R.A. and J.T. Collins (Eds.). McGraw-Hill, NewYork, New York, USA.

Reinert, H.K. 1992. Radiotelemetric field studies of pitvipers: Data acquisition and analysis. Pp. 185-197 *In* Biology of the Pitvipers. Campbell, J.A. and E.D. Brodie Jr., (Eds.). Selva, Tyler, Texas, USA.

Reinert, H.K. 1993. Habitat selection in snakes. Pp. 201-240 *In* Snakes: Ecology and Behavior. Seigel R.A. and J.T. Collins (Eds.). McGraw-Hill, New York, New York, USA.

Saunders D.A., R.J. Hobbs and C.R. Margules. 1991. Biological consequences of ecosystem fragmentation: A review. Conservation Biology 5:1-15.

SPSS, Inc. 2003. SPSS Base 12.0 for Windows User's Guide. SPSS Inc., Chicago, Illinois, USA.

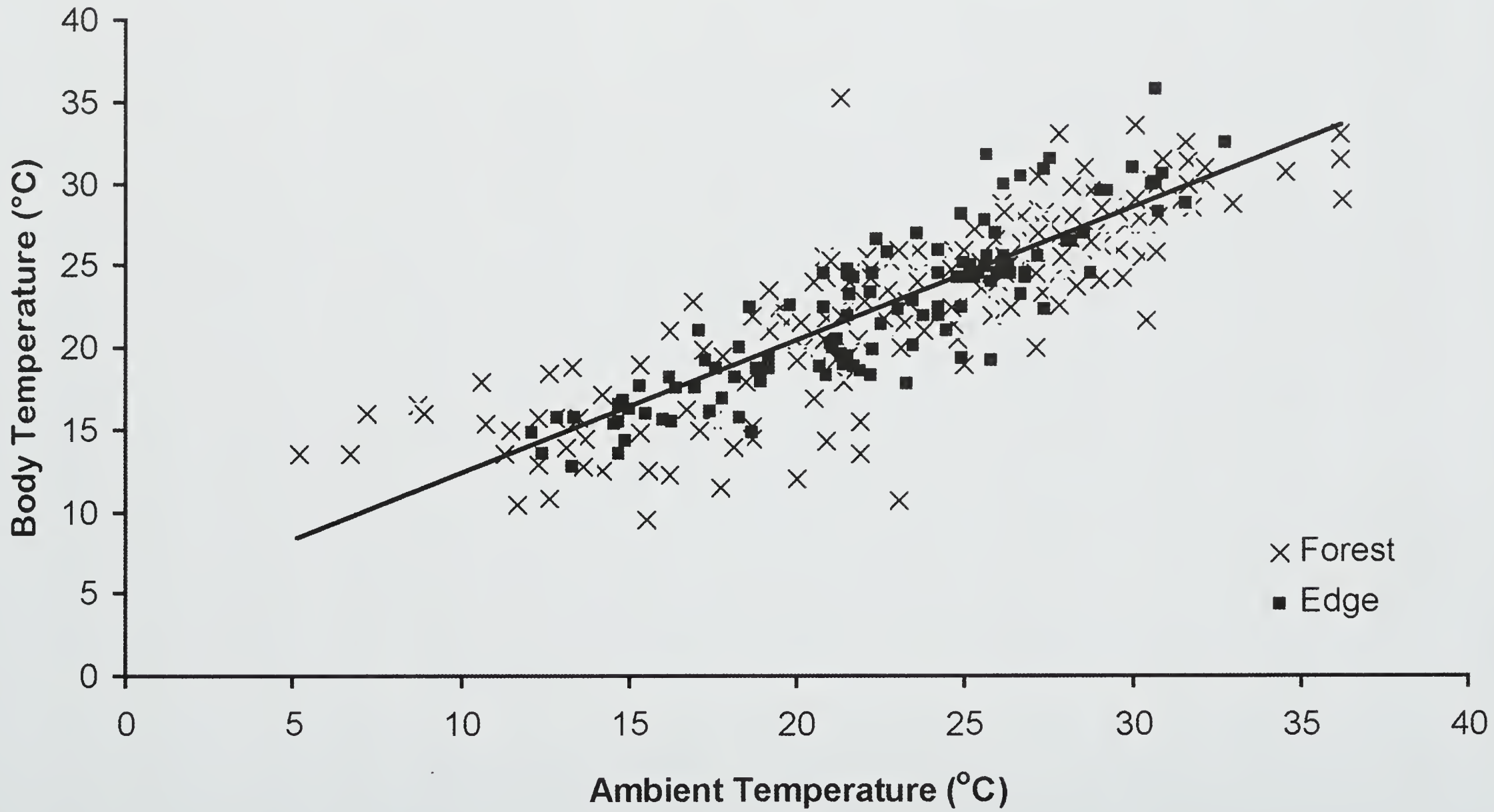
Weatherhead, P.J., and M.B. Charland. 1985. Habitat selection in an Ontario population of the snake, *Elaphe obsoleta*. Journal of Herpetology 19:12-19.

Yahner, R.H. 1988. Changes in wildlife communities near edges. Conservation Biology 2:333-339.

Table 1. Mean (± 1 SE) ambient temperature (°C), snake body temperature (°C), and thermoregulatory effectiveness ($d_a - d_b$) of six male Central Ratsnakes (*Elaphe spiloides*) radiotracked in forest and edge habitat in Clark County, Illinois between 20 May 2003 and 7 November 2004.

Habitat Type	Ambient Temperature (°C)	Subject Body Temperature (°C)	Thermoregulatory Effectiveness
Forest	23.3 ± 0.5	22.8 ± 0.4	0.03 ± 0.23
Edges	22.6 ± 0.4	22.4 ± 0.5	-0.31 ± 0.20

Fig. 1. Body temperature as a function of ambient temperature (°C) for six male Central Ratsnakes (*Elaphe spiloides*) radio-tracked between 20 May 2003 and 7 November 2004 in Clark County, Illinois ($r^2 = 0.73$, $p < 0.001$).



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Breeding Birds and Nest Productivity at Green Wing Environmental Laboratory, Northcentral, Illinois

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ABSTRACT

Green Wing Environmental Laboratory (GWEL) is a 170 ha biological field station in northcentral Illinois that is composed of small woodlots, edge habitat, and wetlands, and is found in a landscape dominated by agriculture and fragmented ecosystems. Animal populations at this site may benefit from a relatively high level of habitat diversity. Alternatively, previous studies have demonstrated that birds breeding in small habitat patches surrounded by agriculture experience low reproductive success due to high rates of nest predation and Brown-headed Cowbird (*Molothrus ater*) brood parasitism. We conducted three studies aimed at assessing the reproductive success of birds at GWEL. Study 1 assigned breeding status using the methodology of the Breeding Bird Atlas. We observed 124 species, 97 of which showed evidence of breeding: 66 confirmed, 14 probable, and 17 possible. In Study 2, we estimated abundance of summer birds and found that the Red-winged Blackbird (*Agelaius phoeniceus*), Song Sparrow (*Melospiza melodia*), and Gray Catbird (*Dumetella carolinensis*) were present in the highest numbers. In Study 3, an analysis of nest productivity, daily nest survival (DNS) was high for two edge-breeding species, Red-winged Blackbird (DNS = 0.92±0.016) and Gray Catbird (DNS = 0.96±0.01), which included no evidence of nest parasitism from the Brown-headed Cowbird. These studies suggest that edge species are common at GWEL and may experience high reproductive success. Future work should examine nest productivity of other edge species, forest breeders, e.g., Wood Thrush (*Hylocichla mustelina*) and Ovenbird (*Seiurus aurocapilla*), and species of conservation concern, e.g., Field Sparrow (*Spizella pusilla*).

INTRODUCTION

Green Wing Environmental Laboratory (GWEL) is a biological field station in the Prairie Peninsula Physiographic Area (Fitzgerald et al. 2000) of northcentral Illinois. In the early 1800's, GWEL was embedded in a landscape dominated by prairie and was part of a ~6,000 ha forest, which included isolated wetlands (INHS 2006). Agricultural development post-European settlement has reduced prairie, forest, and wetlands by an estimated 99.9%, 50%, and 91%, respectively (Levin 2000, IILCP 2004, INHS 2006). In the 1930's, the site was managed for row crops (mixed use grasslands), although many small forest patches were present. Despite little management since this time, mature forest area

has increased in size by 62 ha, mixed use grassland area has decreased by 86 ha, and wetlands have become reestablished. Current land use in the immediate vicinity of GWEL includes Conservation Reserve Program (CRP) grasslands, small woodlots and hedgerows, and agriculture. In addition, recent exurban development immediately adjacent to this site has resulted in four new single family residences, which has converted mature forest to edge/early successional habitat.

Today, GWEL consists of 170 ha of forest fragments [black and bur oak, hickory, and walnut estimated at 42 ha based on "core" area in Burke and Nol (2000)], white pine plantations (12 ha), wet meadows (21 ha), old fields and a reconstructed prairie (combined 17 ha), small pot-holes, and streams. The reminder of the site (78 ha) may effectively function as habitat edge, which negatively affects fitness in breeding birds compared to conspecifics in large habitat patches. For example, brood parasitism by the Brown-headed Cowbird (*Molothrus ater*) and nest predation may be high in small woodlots and grasslands resulting in reduced nest productivity (Burke and Nol 2000; Herkert et al. 2003). Small forest patches may also act as sinks for birds, such as Ovenbird (*Seiurus aurocapilla*), Wood Thrush (*Hylocichla mustelina*), Veery (*Catharus fuscescens*), and Rose-breasted Grosbeak (*Pheucticus ludovicianus*), in a metapopulation context (Nol et al. 2005). Donovan et al. (1997) found that birds breeding in early successional and forest edge habitats experience higher nest-depredation rates than in "core" forest habitats. In addition, highly and moderately fragmented sites experience relatively high nest predation rates due to mammalian (raccoon, opossum, and canid) and avian predators. Much of GWEL is immediately surrounded by row crop development, which has indirect negative effects on nest productivity (Heske et al. 2001). Increasing exurban development at the eastern and southern boundaries of the site may reduce survival and reproduction of native birds near homes (Hansen et al. 2005). In addition, as exurban housing density increases, there is a tendency for 1) early successional habitat (edge) to increase, 2) native species richness to decrease, and 3) an increase in abundance of exotic predators (domestic dogs and cats) and human-adapted natives [Brown-headed Cowbird and American Robin (*Turdus migratorius*)].

Previous field studies at GWEL documented 55 summer species and abundance was highest in the Red-winged Blackbird (*Agelaius phoeniceus*), Blue Jay (*Cyanocitta cristata*), and American Crow (*Corvus brachyrhynchos*) (McKay and Hager 2005). This information offers limited insight about the population status of breeding birds. A deeper understanding of the status and short-term demography may be derived from the details of breeding and nest success (Martin 1992, Martin and Geupel 1993, Faaborg 2002). We conducted three field studies to assess those characteristics in the birds of GWEL. In Study 1, we identified species as confirmed, probable, and possible breeders using the methodology of the Breeding Bird Atlas (BBA) (Smith 1990). Although the Illinois BBA was recently published (Kleen et al. 2004), field surveys were completed in 1991 and may not have examined forested areas in the region since this habitat is not well represented. In Study 2, we estimated the abundance and richness of summer species using standardized transects. In Study 3, we quantified breeding productivity via daily nest survival in birds known to reproduce in edge habitat.

METHODS

Study 1

We assessed the likelihood of breeding in the birds of GWEL from late-May through August 2005-2007. The methods were generally consistent with the Breeding Bird Atlas (BBA), which recommends the use of reproductive behavior to assign standardized breeding categories: confirmed, probable, possible, and observed; however, in Appendix 1 we explain how these were synthesized from Smith (1990) and Cutright et al. (2006). We completed approximately one 7-h survey/week, which began at sunrise. Our basic objectives were to document evidence of breeding in at least 75% of species observed and, of these, to confirm at least 50% (Cutright et al. 2006). We also report the full repertoire of breeding classification and codes observed for each species; this provides more detailed and valuable natural history information which is important since GWEL is relatively new (established in 1991). In 2005 and 2007, we conducted BBA surveys in March (2 visits; 9 field-h), April (8 visits; 30 field-h), and May (3 visits; 7 field-h) for early breeding species. Twelve nocturnal BBA surveys (22 field-h) were conducted in 2005-2007 for the American Bittern (*Botaurus lentiginosus*), Least Bittern (*Ixobrychus exilis*), Virginia Rail (*Rallus limicola*), Sora (*Porzana carolina*), American Woodcock (*Scolopax minor*), Eastern Screech-Owl (*Megascops asio*), Barred Owl (*Strix varia*), Common Nighthawk (*Chordeiles minor*), and Whip-poor-will (*Caprimulgus vociferous*). We would occasionally broadcast recordings of bitterns, rails, and owls to elicit responses (McGarigal and Fraser 1985, Ritchison et al. 1988, Conway 2005). Seven artificial nest boxes (entrance hole diameter ~40 mm), placed at the edges (<100 m) of grasslands and wetlands in 2003, were monitored throughout the study.

Poole et al. (1992) and Baicich and Harrison (1997) were consulted for identification of nests, eggs, nestlings, and breeding behavior. Common and scientific names used throughout follow the American Ornithologists' Union (1998). We make qualitative comparisons between our results and the data in surrounding Illinois BBA blocks (Amboy, Dixon East, Franklin Grove, Ashton, Sublette, Mendota West, La Moille, Ohio, and Walton) (Kleen et al. 2004, Breeding Bird Atlas Explorer 2007).

Study 2

In June and July 2007, we estimated abundance of summer birds from 6 fixed-width strip transects (Bibby et al. 2000; 100 m on each side of transect), which averaged (±1SD) 1.9 h (±0.51) and 1.4 km (±0.35) in length. We systematically placed transects so that a representative sample of the site's habitats was obtained. Surveys could not be confined within a habitat because the site is highly fragmented and contains many small habitat patches. Each transect was completed once during favorable weather (PWRC 2001). This survey methodology allowed us to evaluate relative commonness and diversity. We used estimates to categorize species as Abundant (≥6 birds/h), Common (2.00-5.99 birds/h), Fairly Common (1.00-1.99 birds/h), and Rare (<1.00 birds/h and birds documented at times other than during surveys) (Andres et al. 2004). We make qualitative comparisons to previously recorded abundance estimates from the Troy Grove (#68) Breeding Bird Survey route, which was ~19 km south of and the closest route in proximity to GWEL (PWRC 2007).

Study 3

From late May-July 2007, we located and monitored the nests of seven species known to breed in edge habitat: Tree Swallow (*Tachycineta bicolor*), Eastern Bluebird (*Sialia sialis*), Gray Catbird (*Dumetella carolinensis*), Brown Thrasher (*Toxostoma rufum*), Yellow Warbler (*Dendroica petechia*), Northern Cardinal (*Cardinalis cardinalis*), and Red-winged Blackbird (Yasukawa and Searcy 1995, Imbeau et al. 2003, McKay and Hager 2005). We focused on these species since previous work suggested that they were abundant relative to forest-nesting species, such as Ovenbird and Wood Thrush (McKay and Hager 2005).

We located nests by visual inspection of vegetation and adult distress calls, which is an indicator of a nearby nest (Burhans and Thompson 2006). Nests were identified by parent, nest, chick, or egg characteristics (Baicich and Harrison 1997, Sibley 2003, DeVore et al. 2004). We marked nests with a piece of pink plastic flagging (~15 cm) at a distance 5 m to the north of the nest (Johnson and Temple 1990, Galligan et al. 2006) and recorded latitude/longitude via GPS, nest height, and plant genus in which a nest was found. In addition, we monitored breeding in seven nest boxes mentioned previously.

We monitored nests every 3 to 4 days after the initial marking (Martin and Geupel 1993, Brawn 2006). Welfare impacts to nests were minimized by approaching them from different pathways (which would not dead end at the nest) and by minimizing damage to surrounding vegetation. Whenever possible, we visually examined nests using a hand mirror and a pole-mounted mirror (~2.5 m in length), which minimized disturbance.

We recorded nests as successful based on appropriate timing of chick development, intact construction of the nest, presence of fledglings off of nest, fecal sacs in a nest, and flattened edges of the nest, and produced at least one fledgling (Martin and Geupel 1993). Nests were considered depredated if damage to nest construction and remnants of eggs, egg shells, and chicks were observed in or around nests. The timing of nest success or depredation was placed at a date halfway between the previous and final monitoring visits (Johnson and Temple 1990).

Daily nest survival (DNS), the probability of a nest surviving with at least one viable chick on any day of the nesting period, was calculated using the Mayfield Method (Johnson 1979). A minimum of 20 nests per species was required for meaningful estimates of DNS (Martin and Geupel 1993), which was calculated for the Gray Catbird and Red-winged Blackbird. Parasitism by the Brown-headed Cowbird for each species was recorded as the percent of nests parasitized. We used a chi-squared test to evaluate differences in nest location (plant genus) for the Gray Catbird and Red-winged Blackbird. This analysis was restricted only to data gathered in an opportunistic sense; we made no attempt to identify plants available to, but not used, by birds.

We make general statements about regional population status through the use of maintenance fecundity, which may be considered as the annual recruitment of young that balances adult mortality (Burke and Nol 2000). This was calculated using estimates of lifespan and survivorship reported in the literature (Yasukawa and Searcy 1995, DeSante et al. 2001, de Magalhaes et al. 2005). We reasoned that if our estimates of the # offspring/year was greater than maintenance fecundity, then nest productivity at GWEL

contributed to population numbers in 2007. Alternatively, if these estimates were less than maintenance fecundity, then GWEL recruitment failed to add to the regional population. Modeling population trends, per se, was not an objective of our work, and, thus, we make only general conclusions in this context.

RESULTS

Study 1

We observed 124 species, 97 of which showed evidence of breeding at GWEL: 66 confirmed, 14 probable, and 17 possible (Tables 1-4). Standardized behaviors of Singing, Territoriality, Pair, Feeding Young, Fledged Young, and Nests with Eggs were observed often (≥50%) in confirmed breeders (see Appendix 1 for breeding code definitions). We confirmed 25 species not reported as such in the Amboy and surrounding Illinois BBA blocks (Kleen et al. 2004), including Wild Turkey (*Meleagris gallopavo*), Red-bellied Woodpecker (*Melanerpes carolinus*), Veery, Wood Thrush, Ovenbird, and Field Sparrow (*Spizella pusilla*) (Table 1). In contrast, we failed to observe five species that were confirmed by Kleen et al. (2004) in these BBA blocks: Mute Swan (*Cygnus olor*), Gray Partridge (*Perdix perdix*), Horned Lark (*Eremophila alpestris*), Savannah Sparrow (*Passerculus sandwichensis*), and Western Meadowlark (*Sturnella neglecta*).

We documented nest parasitism in 11 species (Table 5) and nest depredation in: Canada Goose (*Branta canadensis*), Eastern Kingbird (*Tyrannus tyrannus*), White-eyed Vireo (*Vireo griseus*), Veery, American Robin, Yellow Warbler, Eastern Towhee (*Pipilo erythrophthalmus*), Northern Cardinal, Indigo Bunting (*Passerina cyanea*), and Red-winged Blackbird. In 2005, a Red-winged Blackbird was observed to consume the first and only egg within a Yellow Warbler nest, after which the female warbler disassembled the nest and, using the same nest material, re-built another nest ~15 m away (A. Wenmacher, unpubl. data).

For the nests we monitored (see Tables 1 and 2), the following observations were at the edge of or beyond known egg or nestling dates for this region: 1) Sora nest with eggs on 23 July 2006; 2) Eastern Phoebe nest with eggs on 7 July 2006; 3) probable re-nest attempt for single-brooded Yellow-throated Vireo, with male and female pair observed making nest on 7 July 2006; and 4) nest with nestlings of Eastern Towhee on 7 September 2005 (Weeks 1994; Greenlaw 1996; Melvin and Gibbs 1996; Rodewald and James 1996).

Study 2

We observed 72 species during abundance surveys: 8 Abundant, 24 Common, 24 Fairly Common, and 16 Rare (Tables 1, 3 and 4; Appendix 2). Dickcissel (*Spiza americana*), Grasshopper Sparrow (*Ammodramus savannarum*), and Rock Pigeon (*Columba livia*) were observed in habitats immediately adjacent to GWEL. We encountered a male Orchard Oriole (*Icterus spurius*) shortly after the end of one survey route on 12 June 2007.

Study 3

We calculated reliable estimates (≥20 nests) of DNS for the Red-winged Blackbird and Gray Catbird (Table 6). Nest loss for all species was attributed to predation, except for

the Red-winged Blackbird in which 13 of 25 nests were lost to flooding after heavy rainfall. Nest loss for the Gray Catbird, Northern Cardinal, and Red-winged Blackbird, was higher for nests with eggs than nests with nestlings. For the Yellow Warbler and Brown Thrasher, we found equal proportions of depredated nests for those containing eggs and those with nestlings. For nest boxes, one depredated Tree Swallow nest resulted in the loss of chicks and adults. House Sparrows (*Passer domesticus*) quickly began nest building on top of the swallow nest, which contained the bodies of both adult swallows.

We observed no direct cause of nest predation for Study 3, although many predators are known from the site, e.g., raccoon (*Procyon lotor*), American mink (*Neovision vision*), squirrels (*Sciurus* spp.), birds, and snakes [northern watersnake (*Nerodia sipedon*), western ribbonsnake (*Thamnophis proximus*), and common gartersnake (*Thamnophis sirtalis*)]. During this study, only one nest (Yellow Warbler) was parasitized by the Brown-headed Cowbird (Table 5). (Parasitized nests of other species reported in Table 5 were observed during Study 1.) A Tree Swallow nest was parasitized by an Eastern Bluebird, although the bluebird egg was not brooded.

Gray Catbirds nested in *Cornus* (N = 11 nests), *Lonicera* (N = 5), and *Rubus* (N = 6), but proportions were not significantly different (χ^2=2.68, DF=2, P=0.26). Significantly more Red-winged Blackbird nests (N = 24) were found in *Phalaris* grass (χ^2=13.54, DF=1, P=0.0002) than in other plants (N = 5, which was a combined group of three plant genera in order to meet assumptions of the test).

DISCUSSION

We set out to document evidence of breeding in at least 75% of species observed and, of these, to confirm at least 50% (Cutright et al. 2006). We achieved these objectives with 78% (97/124) documented evidence and 68% (66/97) confirmed breeders. Probable breeders were easily heard, but in some cases, difficult to visually locate, e.g., American Woodcock. This was exacerbated for the Whip-poor-will, which was heard in all years except 2007. Moreover, we observed the Yellow-breasted Chat (*Icteria virens*) and Eastern Screech-Owl only in 2005. Possible breeders included species that were difficult to confirm because of secretive breeding habits, such as Blue-winged Teal (*Anas discors*), or they were observed infrequently, e.g., Purple Martin (*Progne subis*). To our knowledge we are the first to document depredation on a Yellow Warbler nest by the Red-winged Blackbird and one of the few to report the observation of the female warbler using material from the depredated nest to construct a new one (Lowther et al. 1999).

This and previous work on the birds at GWEL (McKay and Hager 2005) documented (1) 26 summer species considered threatened, declining, or rare at the continental and national scales, (2) 22 priority and declining species in the Prairie Peninsula physiographic area, and (3) six state species of special concern (Fitzgerald et al. 2000, IESPB 2006, Butcher and Niven 2007, Butcher et al. 2007, Sauer et al. 2007). Seven confirmed or probable breeders [Northern Bobwhite (*Colinus virginianus*), Whip-poor-will, Red-headed Woodpecker (*Melanerpes erythrocephalus*), Field Sparrow, Grasshopper Sparrow, Dickcissel, and Eastern Meadowlark (*Sturnella magna*)] are of conservation concern at national and physiographic levels (Fitzgerald et al. 2000, Butcher et al. 2007, Sauer et al. 2007).

Abundance of breeding birds in 2007 was highest in the Red-winged Blackbird, Song Sparrow (*Melospiza melodia*), and Gray Catbird (Appendix 2). These results may more accurately reflect abundance at this site since McKay and Hager (2005) conducted only one point count survey-day during the summer. The Common Yellowthroat (*Geothlypis trichas*) was abundant at GWEL despite a significant average decline of 0.8%/yr in 1966-2006 from BBS work in the Prairie Peninsula physiographic area (Sauer et al. 2007). Results from the closest BBS route, Troy Grove (#68), show that abundance was highest in the Red-winged Blackbird, Common Grackle, and American Robin (PWRC 2007). Although close in proximity, overall differences in richness between sites appear to correspond to the habitats assessed: woodlands, wetlands, grasslands, and edge at GWEL and (presumably) open farmland and rural towns for the BBS route.

The BBS population assessment for the Red-winged Blackbird in the Prairie Peninsula physiographic area reported no significant difference in annual percent change in the number of individuals for 1966-2006 (Sauer et al. 2007). This suggests that reproductive success in local areas was high enough to maintain regional demographic stability. The DNS calculated for this species at GWEL in 2007 was similar to DNS estimates reported elsewhere in the Midwest (Galligan et al. 2006). However, the #offspring/year was only ~50% of the maintenance fecundity for this species (Table 6). This implies that annual recruitment from GWEL failed to contribute new individuals into the population. Nest submersion accounted for over half of the nest losses due to heavy rain events in the summer months. Nesting over water is reported for this species and it may minimize predation risk relative to nests on land (Yasukawa and Searcy 1995). During years of average rainfall nest productivity at GWEL may be high enough to contribute more positively to the regional demography of Red-winged Blackbirds.

We observed no evidence of nest parasitism for the Red-winged Blackbird at GWEL. The incidence of parasitism in this species is reported at 30% across habitats (Galligan et al. 2006). Levels of parasitism may be inversely correlated with high breeding density of adults, which was documented at this site in Study 2, and may be reduced by limited cowbird egg recognition and subsequent removal (Yasukawa and Searcy 1995).

The BBS also reported no significant difference in annual percent change in the number of Gray Catbirds for 1966-2006 (Sauer et al. 2007). Daily nest survival in 2007 was high and was similar to other studies in the region, although year-to-year reproductive success may vary widely (Cimprich and Moore 1995). Moreover, our estimate of the #offspring/year was higher than maintenance fecundity. This suggests that recruitment from GWEL in 2007 may have contributed to the regional population.

Low nest predation for the Gray Catbird may be attributed to several factors. Catbird nests are constructed deep within the shrub interior, which may minimize conspicuousness to predators (Cimprich and Moore 1995). Plant genera (*Cornus*, *Rubus*, and *Lonicera*) used by catbirds at GWEL for nesting match the genera reported elsewhere (Cimprich and Moore 1995). Additionally, aggressive parental behavior in this species may also confer protection of active nests (Cimprich and Moore 1995). Low incidence of nest parasitism was expected since catbirds can recognize and remove cowbird eggs from nests (Cimprich and Moore 1995).

Tree Swallows and Eastern Bluebirds experienced relatively high nest success. One Tree Swallow nest was depredated most probably by House Sparrows (K. K. Hallinger, pers. comm.). Robertson et al. (1992) report that both sexes of Tree Swallows often grapple with avian intruders inside nest cavities. Combatant Tree Swallows have been found injured or dead inside boxes, which we observed, or on the ground after such fights. Intraspecific nest building on top of dead adults has been documented for the White-throated Swift (*Aeronautes saxatalis*) (Rett 1946), but not in an interspecific context (House Sparrows nesting on dead Tree Swallows). Moreover, our observations included recently killed Tree Swallows, whereas the adult swift appeared to die the year prior to being found.

Nest parasitism by Eastern Bluebirds is rare, but known hosts include Carolina Wren (*Thryothorus ludovicianus*), chickadees, and House Sparrows (Gowaty and Plissner 1998). Only one of ~400 monitored Tree Swallow nests in Virginia was parasitized by bluebirds (K. K. Hallinger, unpubl. data). To our knowledge, this is the first published documentation of bluebirds parasitizing a Tree Swallow nest.

Results of Studies 1-3 suggest that GWEL is an important site for breeding birds, although reproductive success appeared to vary among species when viewed along with the abundance data, nest productivity, and anecdotal observations of nest parasitism. Reasons for this may be related to the biological characteristics of the site, including fragmented habitats (Burke and Nol 2000, Herkert et al. 2003, Hansen et al. 2005), many of which are immediately adjacent to developed agricultural land and are known to increase the risk of nest predation and parasitism (Heske et al. 2001). Moreover, high inter-annual variation in weather may have affected our results. In 2004-2007, above average temperatures were recorded in Illinois, except in 2006, when temperatures were much higher than average (NCDC 2008). Precipitation was near or above average in all years, but not in 2005, when Illinois was characterized at much below average (NCDC 2008). This resulted in "extreme" and "severe" drought conditions in northern Illinois in 2005 and 2006, respectively (NCDC 2008). Qualitatively, wetlands in June-August at GWEL were dry in 2005, temporarily filled with water in 2006, and filled in 2007.

SUMMARY

We evaluated the breeding birds at Green Wing Environmental Laboratory (GWEL), which supports small fragments of forest, wetlands, and grasslands. Field work focusing on atlasing birds (Study 1) identified 124 species, 97 of which showed evidence of breeding: 66 confirmed, 14 probable, and 17 possible. In Study 2, coarse estimates of abundance in 2007 suggested that the Red-winged Blackbird, Song Sparrow, and Gray Catbird were present in the highest numbers. In Study 3, an analysis of nest productivity, daily nest survival (DNS) was generally high for two edge-breeding species, Red-winged Blackbird (DNS = 0.92±0.016) and Gray Catbird (DNS = 0.96±0.01), which included no evidence of nest parasitism by the Brown-headed Cowbird. Future work should examine nest productivity of other edge species, forest breeders, e.g., Wood Thrush and Ovenbird, and species of conservation concern, e.g., Field Sparrow.

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LITERATURE CITED

American Ornithologists' Union. 1998. *Check-list of North American Birds*. Seventh Edition. American Ornithologists' Union. Washington, D.C. USA.

Andres, B. A., M. J. Stotts, and J. M. Stotts. 2004. Breeding birds of Research Natural Areas in southeastern Alaska. Northwestern Naturalist 85:95-103.

Baicich, P. J., and C. J. O. Harrison. 1997. *A Guide to the Nests, Eggs, and Nestlings of North American Birds*, 2nd Edition. Academic Press. San Diego, CA.

Bibby, C. J., N. D. Burgess, D. A. Hill, and S. Mustoe. 2000. *Bird Census Techniques*. Second Edition. Academic Press. London.

Brawn, J. D. 2006. Effects of restoring oak savannas on bird communities and populations. Conservation Biology 20:460-469.

Breeding Bird Atlas Explorer. 2007. U. S. Geological Survey Patuxent Wildlife Research Center & National Biological Information Infrastructure. Available from: http://www.pwrc.usgs.gov/bba. Accessed: 6 November 2007. Data extracted from: Kleen, V. M., L. Cordle, and R. A. Montgomery. 2004. *The Illinois Breeding Bird Atlas*. Illinois Natural History Survey Special Publication No. 26. Champaign, IL.

Burhans, D. E., and F. R. Thompson. 2006. Songbird abundance and parasitism differ between urban and rural shrublands. Ecological Applications 16:394-405.

Burke, D. M., and E. Nol. 2000. Landscape and fragment size effects on reproductive success of forest-breeding birds in Ontario. Ecological Applications 10:1749-1761.

Butcher, G.S., and D.K. Niven. 2007. Combining data from the Christmas Bird Count and the Breeding Bird Survey to determine the continental status and trends of North American birds. National Audubon Society, New York NY. Available from: http://www.audubon.org/bird/stateofthebirds/CBID/report.php. Accessed 20 November 2007.

Butcher, G. S., D. K. Niven, A. O. Punjabi, D. N. Pashley, and K. V. Rosenberg. 2007. The 2007 Watchlist for United States Birds. American Birds 61:18-25.

Cimprich, D. A., and F. R. Moore. 1995. Gray Catbird (*Dumetella carolinensis*). The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/167. Accessed: 1 June 2007.

Conway, C. J. 2005. *Standardized North American Marsh Bird Monitoring Protocols*. Wildlife Research Report #2005-04. U.S. Geological Survey, Arizona Cooperative Fish and Wildlife Research Unit. Tucson, AZ.

Cutright, N. J., B. R. Harriman, and R. W. Howe. 2006. *Atlas of the Breeding Birds of Wisconsin*. The Wisconsin Society for Ornithology. Waukesha, WI.

de Magalhaes, J. P., J. Costa, and O. Toussaint. 2005. HAGR: the Human Ageing Genomic Resources. Nucleic Acids Research 33(Database Issue):D537-D543.

DeSante, D. F., M. P. Nott, and D. R. O'Grady. 2001. Identifying the proximate demographic cause(s) of population change by modeling spatial variation in productivity, survivorship, and population trends. ARDEA 89(special issue):185-208.

DeVore, S., S. Bailey, and G. Kennedy. 2004. *Birds of Illinois*. Lone Pine Publishing, Auburn, WA.

Donovan, T. M., P. W. Jones, E. M. Annand, and F. R. Thompson III. 1997. Variation in local-scale edge effects: mechanisms and landscape context. Ecology 78:2064-2075.

Fitzgerald, J. A., J. R. Herkert, and J. D. Brawn. 2000. Partners in Flight bird conservation plan for the Prairie Peninsula (Physiographic Area 31). Available from: http://www.blm.gov/wildlife/plan/pl_31_10.pdf. Accessed: 10 October 2005.

Faaborg, J. 2002. *Saving Migrant Birds: Developing Strategies for the Future*. University of Texas Press, Austin, TX.

Galligan, E. W., T. L. DeVault, and S. L. Lima. 2006. Nesting success of grassland and savanna birds on reclaimed surface coal mines of the Midwestern United States. Wilson Journal of Ornithology 118:537-546.

Gowaty, P. A., and J. H. Plissner. 1998. Eastern Bluebird (*Sialia sialis*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/381. Accessed: 23 March 2008.

Greenlaw, J. S. 1996. Eastern Towhee (*Pipilo erythrophthalmus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/262. Accessed: 23 March 2008.

Hansen, J. H., R. L. Knight, J. M. Marzluff, S. Powell, K. Brown, P. H. Gude, and K. Jones. 2005. Effects of exurban development on biodiversity: patterns, mechanisms, and research needs. Ecological Applications 15:1893-1905.

Herkert, J. R., D. L. Reinking, D. A. Wiedenfeld, M. Winter, J. L. Zimmerman, W. E. Jensen, E. J. Finck, R. R. Koford, D. H. Wolfe, S. K. Sherrod, M. A. Jenkins, J. Faaborg, and S. K. Robinson. 2003. Effects of prairie fragmentation on the nest success of breeding birds in the Midcontinental United States. Conservation Biology 17:587-594.

Heske, E. J., S. K. Robinson, and J. D. Brawn. 2001. Nest predation and neotropical migrant songbirds: piecing together the fragments. Wildlife Society Bulletin 29:52-61.

[IESPB] Illinois Endangered Species Protection Board. 2006. *Endangered and Threatened Species List*. Available from: http://dnr.state.il.us/espb/datelist.htm. Accessed: 8 January 2008.

[IILCP] Illinois Interagency Landscape Classification Project. 2004. *Land Cover of Illinois Statistical Summary, 1999-2000*. Available from: http://www.agr.state.il.us/gis/stats/landcover/index.htm. Accessed: 4 February 2008.

[INHS] Illinois Natural History Survey. 2006. *Land Cover of Illinois in the Early 1800's*. Available from: http://www.inhs.uiuc.edu/cwe/maps/glo.html. Accessed: 4 February 2008.

Imbeau, L, P. Drapeau, and M. Mönkkönen. 2003. Are forest birds categorized as "edge species" strictly associated with edges? Ecography 26:514-520.

Johnson, D. H. 1979. Estimating nest success: The Mayfield method and an alternative. The Auk 96:651-661.

Johnson, R. G., and S. A. Temple. 1990. Nest predation and brood parasitism of tallgrass prairie birds. Journal of Wildlife Management 54:106-111.

Kleen, V. M., L. Cordle, and R. A. Montgomery. 2004. *The Illinois Breeding Bird Atlas*. Illinois Natural History Survey, Special Publication No. 26. Champaign, IL.

Levin, G. 2000. Restoration. In: Rice, T. and C. Warwick, eds., *Illinois Natural History Survey Annual Report 1998-1999*. Available from http://www.inhs.uiuc.edu/annualreports/98_99/restor.html. Accessed on 2 September 2003.

Lowther, P. E., C. Celada, N. K. Klein, C. C. Rimmer and D. A. Spector. 1999. Yellow Warbler (*Dendroica petechia*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/454. Accessed: 23 March 2008.

Martin, T. E. 1992. Breeding Productivity Considerations: What are the appropriate habitat features for management? In: J. M. Hagan III and D. W. Johnston, eds., *Ecology and Conservation of Neotropical Migrant Landbirds*. Smithsonian Institution Press. Washington, DC.

Martin, T. E. 1995. Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monographs 65:101-127.

Martin, T. E., and G. R. Geupel. 1993. Nest monitoring plots: methods for locating nests and monitoring success. Journal of Field Ornithology 64:507-519.

McGarigal, K., and J. D. Fraser. 1985. Barred Owl responses to recorded vocalizations. The Condor 87:552-553.

McKay, K. J., and S. B. Hager. 2005. An assessment of avian richness and relative abundance at Green Wing Environmental Laboratory, Lee County, Illinois. Meadowlark 14:8-14.

Melvin, S. M., and J. P. Gibbs. 1996. Sora (*Porzana carolina*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/250. Accessed: 23 March 2008.

[NCDC] National Climatic Data Center. 2008. *Weather/Climate Events*. Available from: http://www.ncdc.noaa.gov/oa/climateresearch.html. Accessed: 22 March 2008.

Nol, E., C. M. Francis, and D. M. Burke. 2005. Using distance from putative sources woodlots to predict occurrence of forest birds in putative sinks. Conservation Biology 19:836-844.

[PWRC] Patuxent Wildlife Research Center. 2001. *Instructions for Conducting the North American Breeding Bird Survey*. Available from: www.pwrc.usgs.gov/bbs/participate/instructions.html# ACCEPTABLE. Accessed: 24 January 2008.

[PWRC] Patuxent Wildlife Research Center. 2007. *North American Breeding Bird Survey, Results and Analyses*. Available from: http://www.pwrc.usgs.gov/bbs/results. Accessed: 19 November 2007.

Poole, A., P. Stettenheim, and F. Gill, editors. 1992. *The Birds of North America : Life Histories for the 21st Century*. American Ornithologists' Union, Washington, D.C., Academy of Natural Sciences. Philadelphia, PA.

Rett, E. Z. 1946. An unusual nest of the White-throated Swift. The Condor 48:141.

Ritchison, G., P. M. Cavanagh, J. R. Belthoff, E. J. Sparks. 1988. The singing behavior of Eastern Screech-Owls: seasonal timing and response to playback of conspecific song. The Condor 90:648-652.

Robertson, R. J., B. J. Stutchbury and R. R. Cohen. 1992. Tree Swallow (*Tachycineta bicolor*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/011. Accessed: 23 March 2008.

Rodewald, P. G., and R. D. James. 1996. Yellow-throated Vireo (*Vireo flavifrons*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/247. Accessed: 23 March 2008.

Sauer, J. R., J. E. Hines, and J. Fallon. 2007. *The North American Breeding Bird Survey, Results and Analysis 1966 - 2006*. Version 10.13.2007. USGS Patuxent Wildlife Research Center. Laurel, MD.

Sibley, D. A. 2003. *The Sibley Field Guide to Birds of Eastern North America*. Knopf Publishing Group, New York.

Smith, C. R. 1990. *Handbook for Atlasing North American Breeding Birds*. Available from: http://www.bsc-eoc.org/norac/atlascont.htm. Accessed: 22 January 2008.

Weeks, Jr., H. P. 1994. Eastern Phoebe (*Sayornis phoebe*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/094. Accessed: 23 March 2008.

Yasukawa, K., and W. A. Searcy. 1995. Red-winged Blackbird (*Agelaius phoeniceus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/184. Accessed: 17 December 2006.

Table 1. Abundance and comparison to Kleen et al. (2004) of Confirmed breeders. Asterisks identify species not observed during abundance surveys.

Common Name	Scientific Name	Abundance	Not Confirmed in Kleen et al. (2004)
Canada Goose	*Branta canadensis*	Common	
Wood Duck	*Aix sponsa*	Common	
Mallard	*Anas platyrhynchos*	Fairly Common	
Ring-necked Pheasant	*Phasianus colchicus*	Rare	
Wild Turkey	*Meleagris gallopavo*	Fairly Common	X
Northern Bobwhite	*Colinus virginianus*	Rare*	
Green Heron	*Butorides virescens*	Fairly Common	X
Cooper's Hawk	*Accipiter cooperii*	Rare	X
Red-tailed Hawk	*Buteo jamaicensis*	Rare	
American Kestrel	*Falco sparverius*	Rare*	
Sora	*Porzana carolina*	Rare	X
Killdeer	*Charadrius vociferus*	Abundant	
Mourning Dove	*Zenaida macroura*	Common	
Yellow-billed Cuckoo	*Coccyzus americanus*	Fairly Common	
Great Horned Owl	*Bubo virginianus*	Rare*	
Ruby-throated Hummingbird	*Archilochus colubris*	Rare	X
Red-bellied Woodpecker	*Melanerpes carolinus*	Common	X
Downy Woodpecker	*Picoides pubescens*	Rare	
Hairy Woodpecker	*Picoides villosus*	Fairly Common	X
Northern Flicker	*Colaptes auratus*	Fairly Common	
Eastern Wood-Pewee	*Contopus virens*	Common	X
Willow Flycatcher	*Empidonax traillii*	Rare	
Eastern Phoebe	*Sayornis phoebe*	Rare*	X
Great Crested Flycatcher	*Myiarchus crinitus*	Fairly Common	
Eastern Kingbird	*Tyrannus tyrannus*	Fairly Common	
White-eyed Vireo	*Vireo griseus*	Rare	X
Yellow-throated Vireo	*Vireo flavifrons*	Fairly Common	X
Warbling Vireo	*Vireo gilvus*	Fairly Common	X
Red-eyed Vireo	*Vireo olivaceus*	Common	X
Blue Jay	*Cyanocitta cristata*	Common	
American Crow	*Corvus brachyrhynchos*	Common	
Tree Swallow	*Tachycineta bicolor*	Common	
Black-capped Chickadee	*Poecile atricapillus*	Fairly Common	
White-breasted Nuthatch	*Sitta carolinensis*	Fairly Common	
House Wren	*Troglodytes aedon*	Common	
Sedge Wren	*Cistothorus platensis*	Fairly Common	X
Marsh Wren	*Cistothorus palustris*	Rare	X
Blue-gray Gnatcatcher	*Polioptila caerulea*	Common	X
Eastern Bluebird	*Sialia sialis*	Fairly Common	

Common Name	Scientific Name	Abundance	Not Confirmed in Kleen et al. (2004)
Veery	*Catharus fuscescens*	Fairly Common	X
Wood Thrush	*Hylocichla mustelina*	Fairly Common	X
American Robin	*Turdus migratorius*	Common	
Gray Catbird	*Dumetella carolinensis*	Abundant	
Brown Thrasher	*Toxostoma rufum*	Fairly Common	
European Starling	*Sturnus vulgaris*	Common	
Cedar Waxwing	*Bombycilla cedrorum*	Common	
Yellow Warbler	*Dendroica petechia*	Abundant	
Ovenbird	*Seiurus aurocapilla*	Fairly Common	X
Common Yellowthroat	*Geothlypis trichas*	Abundant	
Scarlet Tanager	*Piranga olivacea*	Rare	X
Eastern Towhee	*Pipilo erythrophthalmus*	Common	X
Chipping Sparrow	*Spizella passerina*	Common	
Field Sparrow	*Spizella pusilla*	Common	X
Lark Sparrow	*Chondestes grammacus*	Rare*	X
Song Sparrow	*Melospiza melodia*	Abundant	
Swamp Sparrow	*Melospiza georgiana*	Abundant	X
Northern Cardinal	*Cardinalis cardinalis*	Common	
Rose-breasted Grosbeak	*Pheucticus ludovicianus*	Common	X
Indigo Bunting	*Passerina cyanea*	Common	
Dickcissel	*Spiza americana*	Fairly Common	
Red-winged Blackbird	*Agelaius phoeniceus*	Abundant	
Common Grackle	*Quiscalus quiscula*	Common	
Brown-headed Cowbird	*Molothrus ater*	Common	
Baltimore Oriole	*Icterus galbula*	Fairly Common	
American Goldfinch	*Carduelis tristis*	Abundant	
House Sparrow	*Passer domesticus*	Fairly Common	

Table 2. Breeding codes of Confirmed breeders. See Appendix 1 for definitions of Breeding Codes and Table 1 for scientific names.

	Breeding Codes															
Common Name	Singing	Pair	Territory	Courtship	Agitated	Building	Carrying Nesting	Nest Building	Distraction Display	Used Nest	Occupied Nest	Fledglings	Fecal Sac	Feeding Young	Nest Eggs	Nest Young
Canada Goose					A						ON				NE	
Wood Duck		P										FL				
Mallard		P										FL				
Ring-necked Pheasant	S		T							UN		FL			NE	
Wild Turkey	S			C					DD			FL			NE	
Northern Bobwhite	S		T									FL				
Green Heron												FL			NE	
Cooper's Hawk		P		C	A				DD			FL		FY		
Red-tailed Hawk					A					UN		FL		FY		NY
American Kestrel											ON	FL				
Sora	S		T									FL		FY	NE	
Killdeer	S											FL				
Mourning Dove	S	P	T		A										NE	
Yellow-billed Cuckoo	S		T									FL		FY	NE	NY
Great Horned Owl	S		T									FL				
Ruby-throated Hummingbird			T				CN									
Red-bellied Woodpecker		P			A						ON	FL		FY		NY
Downy Woodpecker		P			A				DD			FL		FY		NY
Hairy Woodpecker		P			A							FL		FY		NY
Northern Flicker	S	P	T	C								FL		FY		
Eastern Wood-Pewee	S	P	T									FL		FY		
Willow Flycatcher	S	P	T		A									FY		
Eastern Phoebe	S	P	T		A							FL			NE	NY
Great Crested Flycatcher	S	P	T									FL		FY		
Eastern Kingbird	S	P	T		A		CN	NB	DD						NE	
White-eyed Vireo	S	P	T		A									FY	NE	NY
Yellow-throated Vireo	S	P	T				CN	NB				FL		FY		NY
Warbling Vireo	S	P	T		A		CN	NB								
Red-eyed Vireo	S	P	T		A		CN	NB	DD			FL		FY		
Blue Jay	S		T				CN				ON	FL			NE	
American Crow												FL		FY		
Tree Swallow		P		C							ON	FL	FS	FY	NE	NY

	Breeding Codes															
Common Name	Singing	Pair	Territory	Courtship	Agitated	Building	Carrying Nesting	Nest Building	Distraction Display	Used Nest	Occupied Nest	Fledglings	Fecal Sac	Feeding Young	Nest Eggs	Nest Young
Black-capped Chickadee	S		T									FL		FY		
White-breasted Nuthatch	S	P	T								ON			FY		
House Wren	S		T		A				DD					FY	NE	
Sedge Wren	S		T		A	B								FY		
Marsh Wren	S	P	T		A	B									NE	
Blue-gray Gnatcatcher	S	P	T				CN	NB	DD			FL		FY		
Eastern Bluebird	S	P	T	C	A						ON	FL		FY	NE	NY
Veery	S		T		A		CN								NE	
Wood Thrush	S		T		A									FY	NE	
American Robin	S	P	T		A		CN	NB			ON	FL		FY	NE	
Gray Catbird	S	P	T		A		CN					FL		FY	NE	NY
Brown Thrasher	S	P	T		A		CN							FY	NE	NY
European Starling												FL		FY		
Cedar Waxwing	S						CN					FL		FY	NE	
Yellow Warbler	S		T		A		CN	NB		UN		FL		FY	NE	NY
Ovenbird	S	P	T		A							FL		FY	NE	
Common Yellowthroat	S	P	T		A							FL	FS	FY		
Scarlet Tanager	S	P	T				CN					FL		FY		
Eastern Towhee	S	P	T		A				DD	UN		FL		FY		NY
Chipping Sparrow	S	P	T	C	A						ON	FL		FY	NE	
Field Sparrow	S		T		A							FL		FY	NE	NY
Lark Sparrow														FY	NE	NY
Song Sparrow	S		T		A		CN					FL		FY		
Swamp Sparrow														FY		
Northern Cardinal	S	P	T		A							FL		FY	NE	NY
Rose-breasted Grosbeak	S	P	T		A		CN					FL		FY	NE	
Indigo Bunting	S	P	T		A				DD			FL		FY	NE	
Dickcissel	S	P	T									FL		FY		
Red-winged Blackbird	S	P	T	C	A		CN		DD			FL	FS	FY	NE	NY
Common Grackle												FL		FY		
Brown-headed Cowbird	S	P		C								FL		FY	NE	NY
Baltimore Oriole							CN		DD		ON	FL		FY	NE	NY
American Goldfinch	S	P	T				CN		DD		ON	FL		FY	NE	
House Sparrow		P	T				CN	NB		UN			FS	FY	NE	NY

Table 3. Abundance and breeding codes of Probable breeders. Asterisks identify species not observed during abundance surveys. See Appendix 1 for definitions of Breeding Codes.

Common Name	Scientific Name	Abundance	Breeding Codes					
			Singing	Pair	Territory	Courtship	Nest-site	Agitated
Least Bittern	*Ixobrychus exilis*	Rare*	S					
American Woodcock	*Scolopax minor*	Rare*	S		T			
Eastern Screech-Owl	*Megascops asio*	Rare*	S		T			
Barred Owl	*Strix varia*	Rare	S	P	T			
Common Nighthawk	*Chordeiles minor*	Rare*				C		
Whip-poor-will	*Caprimulgus vociferus*	Rare*	S		T			
Chimney Swift	*Chaetura pelagica*	Rare	S					
Carolina Wren	*Thryothorus ludovicianus*	Rare*	S		T			
American Redstart	*Setophaga ruticilla*	Rare*	S		T			
Yellow-breasted Chat	*Icteria virens*	Rare*	S	P	T		N	A
Vesper Sparrow	*Pooecetes gramineus*	Rare*	S					
Grasshopper Sparrow	*Ammodramus savannarum*	Rare	S		T			
Eastern Meadowlark	*Sturnella magna*	Rare*	S					
Orchard Oriole	*Icterus spurius*	Rare*	S		T			

Table 4. Abundance and breeding codes of Possible breeders and species occasionally Observed in late May-July, but which do not breed at GWEL. Asterisks identify species not observed during abundance surveys. See Appendix 1 for definitions of Breeding Classifications.

Breeding Classification	Common Name	Scientific Name	Abundance
Possible	Blue-winged Teal	*Anas discors*	Rare*
	Great Blue Heron	*Ardea herodias*	Fairly Common
	Turkey Vulture	*Cathartes aura*	Fairly Common
	American Coot	*Fulica americana*	Rare*
	Sandhill Crane	*Grus canadensis*	Rare
	Spotted Sandpiper	*Actitis macularius*	Common
	Rock Pigeon	*Columba livia*	Rare
	Black-billed Cuckoo	*Coccyzus erythropthalmus*	Rare*
	Belted Kingfisher	*Megaceryle alcyon*	Rare*
	Red-headed Woodpecker	*Melanerpes erythrocephalus*	Rare*
	Purple Martin	*Progne subis*	Rare*
	Northern Rough-winged Swallow	*Stelgidopteryx serripennis*	Rare*
	Bank Swallow	*Riparia riparia*	Fairly Common
	Cliff Swallow	*Petrochelidon pyrrhonota*	Rare*
	Barn Swallow	*Hirundo rustica*	Fairly Common
	Northern Parula	*Parula americana*	Rare*
	House Finch	*Carpodacus mexicanus*	Rare*
Observed	Green-winged Teal[a]	*Anas crecca*	Rare*
	Double-crested Cormorant[a]	*Phalacrocorax auritus*	Rare*
	Osprey[a]	*Pandion haliaetus*	Rare*
	Red-shouldered Hawk[a]	*Buteo lineatus*	Rare*
	Broad-winged Hawk	*Buteo platypterus*	Rare*
	Semipalmated Plover	*Charadrius semipalmatus*	Rare*
	Solitary Sandpiper	*Tringa solitaria*	Rare*
	Semipalmated Sandpiper	*Calidris pusilla*	Rare*
	Least Sandpiper	*Calidris minutilla*	Rare*
	Baird's Sandpiper	*Calidris bairdii*	Rare*
	Olive-sided Flycatcher	*Contopus cooperi*	Rare*
	Yellow-bellied Flycatcher	*Empidonax flaviventris*	Rare*
	Alder Flycatcher	*Empidonax alnorum*	Rare*
	Least Flycatcher[a]	*Empidonax minimus*	Rare*
	Philadelphia Vireo	*Vireo philadelphicus*	Rare*
	Red-breasted Nuthatch	*Sitta canadensis*	Rare*
	Swainson's Thrush	*Catharus ustulatus*	Rare*
	Tennessee Warbler	*Vermivora peregrina*	Rare*
	Nashville Warbler[a]	*Vermivora ruficapilla*	Rare*
	Magnolia Warbler	*Dendroica magnolia*	Rare*
	Northern Waterthrush	*Seiurus noveboracensis*	Rare*
	Chestnut-sided Warbler[a]	*Dendroica pensylvanica*	Rare*
	Blackburnian Warbler	*Dendroica fusca*	Rare*
	Black-and-white Warbler[a]	*Mniotilta varia*	Rare*
	Mourning Warbler	*Oporornis philadelphia*	Rare*
	Wilson's Warbler	*Wilsonia pusilla*	Rare*
	Canada Warbler	*Wilsonia canadensis*	Rare*

[a]Species was judged as Observed because site does not contain suitable habitat, but future work may describe it as a Possible breeder or higher based on breeding range and behavior.

Table 5. Common names of brood-parasitized species observed in 2005-2007. Most of these data were gathered during Study 1; parasitized nests of the Yellow Warbler and Tree Swallow were documented in Study 3.

Brood Parasite	Species Parasitized	Year(s)	Broods Parasitized	Total Broods Observed
Brown-headed Cowbird	White-eyed Vireo	2007	1	1
	Yellow-throated Vireo	2006, 2007	3	3
	Red-eyed Vireo	2006	2	4
	Yellow Warbler	2007	1	2
	Common Yellowthroat	2007	1	4
	Eastern Towhee	2007	1	2
	Chipping Sparrow	2005, 2007	1	2
	Northern Cardinal	2007	2	7
	Indigo Bunting	2007	2	3
	House Sparrow	2005	2	2
Eastern Bluebird	Tree Swallow	2007	1	4

Table 6. Nest productivity for species monitored in Study 3.

Common Name	Nests observed (# successful)	# Offspring	Offspring/ nest	Offspring/ yr[a]	Maintenance fecundity	ND[b]	DNS[c]	SE[d]
Tree Swallow	4 (3)	-	-	-	-	87	0.989	0.011
Eastern Bluebird	4 (4)	-	-	-	-	72	1	0
Gray Catbird	24 (11)	35	1.46	2.92	1.35	342.5	0.962	0.010
Brown Thrasher	8 (2)	-	-	-	-	93	0.935	0.025
Yellow Warbler	3 (1)	-	-	-	-	45.5	0.956	0.030
Northern Cardinal	3 (0)	-	-	-	-	31.5	0.905	0.052
Red-winged Blackbird	29 (4)	12	0.41	0.83	1.62	295	0.915	0.016

[a]Assuming 2 broods/yr (Martin 1995)
[b]Number of nest days observed
[c]Daily Nest Survival
[d]Standard Error

APPENDIX 1

Breeding classification, codes, and interpretations for Study 1 (Smith 1990, Cutright et al. 2006). We classified birds as Observed, Possible, Probable, or Confirmed breeders. Codes within each category were used to summarize breeding behavior observed during field work and listed in Tables 2-4. Interpretations were used to distinguish among classifications and codes.

Breeding Classification	Breeding Code	Interpretation
Observed	O	A non-breeder or migrant (male or female) *observed* or heard between June 1 and July 31 does not suggest breeding, regardless of habitat. Use this code for species observed in unlikely breeding habitat, out of their normal breeding range, flying over, or with no indication of breeding. This code applies to vultures or raptors flying over, to ducks summering on an urban pond with no breeding habitat, or a heron foraging when no heronry exists in the block. This code records the presence of the species but does not suggest breeding.
Possible	X	A male or female observed in possible suitable nesting habitat within safe dates suggests possible breeding. Note that many species do not have safe dates. Thus, this code can only be used for some species.
Probable	S	*Singing* male detected once in possible suitable nesting habitat indicates probable breeding. If you hear a male of the same species in the same location on another visit determine if code T applies.
	P	*Pair* (male and female) observed in suitable nesting habitat when apparently holding a territory suggests probable breeding. This code is used when it is fairly certain that a mated pair of birds has been observed. Note that two birds of the same species observed together are not always a pair, especially when males and females look alike. In sexually monomorphic species, behavior may indicate a pair.
	T	*Territory* establishment can be based on a singing male observed on at least two different days a week or more apart in the same location. Such repeated observations are a good indication that a bird has taken up residence. Chasing of other birds of the same species often marks a territory and should be recorded using code T. One male American Robin chasing another falls under this code, as would two male owls hooting at each other from opposite sides of a canyon. Caution should be used for some species such as raptors and hummingbirds since they exhibit territorial behaviors in defense of feeding areas and favorite perches while wintering and migrating.
	C	*Courtship* behavior or copulation indicates probable breeding. This code includes courtship displays and food exchanges. Prairie-chickens seen dancing on a lek, hummingbird courtship flights, and the bill tilt or topple-over display of cowbirds would fit this code. Use this code cautiously for ducks and grebes since they often court during migration. For bird banders, this code should be used for females with a brood patch or males with a cloacal protuberance.
	N	Visiting a probable *nest-site* indicates probable breeding when no further breeding evidence is obtained. This code is especially useful for cavity nesters and shrub-nesting species that fly into the same locations and disappear repeatedly. Repeated use of the same probable nest-site must be observed.
	A	*Agitated* behavior or anxiety calls heard from an adult suggests probable breeding. This behavior suggests the probable presence of a nest or young nearby. Do not include agitation that you induce by "pishing" or using taped calls. A goshawk that calls in a distressed fashion falls into this category. If the goshawk swoops at you, you upgrade to the confirmed breeding code DD.

Breeding Classification	Breeding Code	Interpretation
	B	Nest *building* by wrens (Cactus, Bewick's, House, and Marsh), Verdins, or excavation of holes by woodpeckers indicates probable breeding. In Verdins and some species of wrens, unmated males will build nests to attract females. Thus, nests built by these species do not confirm breeding. Also, woodpeckers usually excavate one nest hole and other holes for roosting. Thus, excavation does not confirm breeding in woodpeckers.
Confirmed	PE	*Physiological evidence* of breeding (i.e., highly vascularized incubation (brood) patch or egg in oviduct) based on a bird in hand confirms breeding for bird banders only.
	CN	Birds observed *carrying nesting* material (e.g. sticks, hair, grass, mud, cobwebs) confirms breeding. This applies for all species except for some species of wrens (Cactus, Bewick's, House, March) and Verdins.
	NB	*Nest building* at the actual nest site by all except woodpeckers, Verdins, and wrens, confirms breeding.
	DD	*Distraction display* or injury feigning for defense of an unknown nest or young confirms breeding. This code is used if an adult bird is seen trying to lead people away from a nest or young. A Killdeer giving a "broken wing" act fits this code. The difference between this code and agitated behavior is that the adult bird puts its own life in danger with a distraction display.
	UN	A *used nest* confirms breeding. Caution: This must be carefully identified if it is to be used, and requires a written verification form. Some nests such as those of orioles are persistent and characteristic, but others are more difficult to identify. Be sure that the nest was used during the atlas period. Do not use this code for species that build multiple nests in a breeding season, such as Verdins and Cactus Wrens. Do not collect nests because some species roost in them all year and it is also illegal to collect nests or eggs without a permit.
	ON	Adults entering or leaving a nest site in circumstances indicating an *occupied nest* confirms breeding. This code is not generally used for open-cup nesting birds, unless the nests are high above the ground and the contents cannot be seen. This code should be used mainly for cavity nesting birds that enter a hole and remain inside, leave a hole after having been inside for some time, or for adults that exchange occupancy of a cavity.
	FL	Recent *fledglings* (of altricial species) or downy young (of precocial species such as galliformes, shorebirds or waterfowl) confirm breeding. Fledged young should be incapable of sustained flight. This code does not apply to mobile immatures. This code should be used with caution for species such as starlings and swallows that may move relatively great distances soon after fledging. Use of this code should be used only for recently fledged passerines in the natal areas that are still dependent on parents. A young cowbird begging for food confirms both the cowbird and the host species. If feeding of young by adults is observed use code FY.
	FS	An adult observed carrying a *fecal sac* confirms breeding. Many passerine adults keep their nests clean by carrying away membranous, white fecal sacs.
	FY	*Feeding young*, carrying food for young, or feeding recently fledged young confirms breeding. Be especially careful on the edge of a block. Some birds, such as birds of prey, continue to feed their young long after they've fledged and may move considerable distances. Some birds, such as Common Ravens, may carry food long distances to young in a neighboring block. Also, care should be taken to avoid confusion with courtship feeding, code C.
	NE	A *nest* with *egg(s)*, undisturbed nest with a bird in incubation posture, or eggshells found below the nest confirms breeding. Finding a cowbird egg in a nest is coded NE for both the cowbird and the host. Be careful not to disturb the vicinity of any nests.
	NY	A *nest* with *young* seen or heard confirms breeding. The presence of a cowbird young is coded NY for both cowbird and host species. Caution must be used in approaching nest sites to minimize disturbance. Most confirmations can be accomplished without locating actual nests.

APPENDIX 2

Abundance estimates for Green Wing Environmental Laboratory (72 species) in 2007 and the Troy Grove Breeding Bird Survey (BBS) route #068 (75 species) in 1989-1998 (PWRC 2007).

Green Wing Environmental Laboratory

Common Name	Species	Birds/h
Red-winged Blackbird	*Agelaius phoeniceus*	13.85
Song Sparrow	*Melospiza melodia*	9.61
Gray Catbird	*Dumetella carolinensis*	8.84
Swamp Sparrow	*Melospiza georgiana*	8.4
Common Yellowthroat	*Geothlypis trichas*	7.67
American Goldfinch	*Carduelis tristis*	7.02
Yellow Warbler	*Dendroica petechia*	6.26
Killdeer	*Charadrius vociferus*	6.18
Wood Duck	*Aix sponsa*	5.96
Tree Swallow	*Tachycineta bicolor*	5.81
House Wren	*Troglodytes aedon*	5.18
Cedar Waxwing	*Bombycilla cedrorum*	4.26
Brown-headed Cowbird	*Molothrus ater*	4.02
Field Sparrow	*Spizella pusilla*	3.55
Chipping Sparrow	*Spizella passerina*	3.4
Indigo Bunting	*Passerina cyanea*	3.26
Mourning Dove	*Zenaida macroura*	3.22
American Robin	*Turdus migratorius*	3.14
Eastern Wood-Pewee	*Contopus virens*	3.1
Rose-breasted Grosbeak	*Pheucticus ludovicianus*	3.04
Blue Jay	*Cyanocitta cristata*	3.02
Northern Cardinal	*Cardinalis cardinalis*	2.94
Canada Goose	*Branta canadensis*	2.88
European Starling	*Sturnus vulgaris*	2.61

Troy Grove BBS Route #068

Common Name	Species	Birds/2.5 h
Red-winged Blackbird	*Agelaius phoeniceus*	175.09
Common Grackle	*Quiscalus quiscula*	105.82
American Robin	*Turdus migratorius*	101.09
European Starling	*Sturnus vulgaris*	63
House Sparrow	*Passer domesticus*	61.55
Song Sparrow	*Melospiza melodia*	27.73
Mourning Dove	*Zenaida macroura*	23.45
Western Meadowlark	*Sturnella neglecta*	23
Barn Swallow	*Hirundo rustica*	22.91
Rock Pigeon	*Columba livia*	21.36
American Crow	*Corvus brachyrhynchos*	21.36
Brown-headed Cowbird	*Molothrus ater*	16
Killdeer	*Charadrius vociferus*	15
Chimney Swift	*Chaetura pelagica*	12.18
Vesper Sparrow	*Pooecetes gramineus*	10.09
Horned Lark	*Eremophila alpestris*	7.18
Ring-necked Pheasant	*Phasianus colchicus*	7.09
Northern Cardinal	*Cardinalis cardinalis*	7
American Goldfinch	*Carduelis tristis*	5.36
Brown Thrasher	*Toxostoma rufum*	4.09
Chipping Sparrow	*Spizella passerina*	2.73
Mallard	*Anas platyrhynchos*	2.55
House Finch	*Carpodacus mexicanus*	2.55
Common Yellowthroat	*Geothlypis trichas*	2.36

Green Wing Environmental Laboratory

Common Name	Species	Birds/h
Common Grackle	*Quiscalus quiscula*	2.45
Red-eyed Vireo	*Vireo olivaceus*	2.45
American Crow	*Corvus brachyrhynchos*	2.36
Solitary Sandpiper	*Tringa solitaria*	2.29
Spotted Sandpiper	*Actitis macularius*	2.29
Eastern Towhee	*Pipilo erythrophthalmus*	2.18
Blue-gray Gnatcatcher	*Polioptila caerulea*	2.16
Red-bellied Woodpecker	*Melanerpes carolinus*	2.04
Ovenbird	*Seiurus aurocapilla*	1.9
Warbling Vireo	*Vireo gilvus*	1.66
Great Blue Heron	*Ardea herodias*	1.64
Brown Thrasher	*Toxostoma rufum*	1.63
Eastern Kingbird	*Tyrannus tyrannus*	1.56
Green Heron	*Butorides virescens*	1.56
Bank Swallow	*Riparia riparia*	1.5
Black-capped Chickadee	*Poecile atricapillus*	1.46
White-breasted Nuthatch	*Sitta carolinensis*	1.4
Hairy Woodpecker	*Picoides villosus*	1.38
Veery	*Catharus fuscescens*	1.38
Great Crested Flycatcher	*Myiarchus crinitus*	1.31
Mallard	*Anas platyrhynchos*	1.31
Sedge Wren	*Cistothorus platensis*	1.27
Turkey Vulture	*Cathartes aura*	1.27
Baltimore Oriole	*Icterus galbula*	1.25
Dickcissel	*Spiza americana*	1.25
Northern Flicker	*Colaptes auratus*	1.23
Eastern Bluebird	*Sialia sialis*	1.2
House Sparrow	*Passer domesticus*	1.13
Wild Turkey	*Meleagris gallopavo*	1.12
Yellow-throated Vireo	*Vireo flavifrons*	1.12
Yellow-billed Cuckoo	*Coccyzus americanus*	1.11

Troy Grove BBS Route #068

Common Name	Species	Birds/2.5 h
Indigo Bunting	*Passerina cyanea*	2.36
Gray Catbird	*Dumetella carolinensis*	2.27
Eastern Meadowlark	*Sturnella magna*	2.27
Red-tailed Hawk	*Buteo jamaicensis*	2.18
House Wren	*Troglodytes aedon*	1.91
Dickcissel	*Spiza americana*	1.73
Northern Rough-winged Swallow	*Stelgidopteryx serripennis*	1.64
Baltimore Oriole	*Icterus galbula*	1.64
Eastern Kingbird	*Tyrannus tyrannus*	1.36
Blue Jay	*Cyanocitta cristata*	1.27
Northern Flicker	*Colaptes auratus*	1.18
Great Blue Heron	*Ardea herodias*	1.09
Rose-breasted Grosbeak	*Pheucticus ludovicianus*	0.82
American Kestrel	*Falco sparverius*	0.73
Eastern Bluebird	*Sialia sialis*	0.73
Savannah Sparrow	*Passerculus sandwichensis*	0.73
Red-bellied Woodpecker	*Melanerpes carolinus*	0.64
Warbling Vireo	*Vireo gilvus*	0.64
Canada Goose	*Branta canadensis*	0.55
Northern Bobwhite	*Colinus virginianus*	0.55
American Coot	*Fulica americana*	0.55
Red-headed Woodpecker	*Melanerpes erythrocephalus*	0.55
Bobolink	*Dolichonyx oryzivorus*	0.55
Turkey Vulture	*Cathartes aura*	0.45
Wood Duck	*Aix sponsa*	0.45
Cliff Swallow	*Petrochelidon pyrrhonota*	0.45
Pied-billed Grebe	*Podilymbus podiceps*	0.36
Great Crested Flycatcher	*Myiarchus crinitus*	0.36
Tree Swallow	*Tachycineta bicolor*	0.36
Cedar Waxwing	*Bombycilla cedrorum*	0.36
Downy Woodpecker	*Picoides pubescens*	0.27

Green Wing Environmental Laboratory

Common Name	Species	Birds/h
Wood Thrush	*Hylocichla mustelina*	1.1
Barn Swallow	*Hirundo rustica*	0.94
Chimney Swift	*Chaetura pelagica*	0.94
Scarlet Tanager	*Piranga olivacea*	0.94
Willow Flycatcher	*Empidonax traillii*	0.93
White-eyed Vireo	*Vireo griseus*	0.84
Ring-necked Pheasant	*Phasianus colchicus*	0.81
Downy Woodpecker	*Picoides pubescens*	0.8
Marsh Wren	*Cistothorus palustris*	0.75
Sandhill Crane	*Grus canadensis*	0.75
Sora	*Porzana carolina*	0.75
Cooper's Hawk	*Accipiter cooperii*	0.5
Rock Pigeon	*Columba livia*	0.5
Red-tailed Hawk	*Buteo jamaicensis*	0.44
Ruby-throated Hummingbird	*Archilochus colubris*	0.44
Barred Owl	*Strix varia*	0.25
Grasshopper Sparrow	*Ammodramus savannarum*	0.25
-	-	-
-	-	-
-	-	-

Troy Grove BBS Route #068

Common Name	Species	Birds/2.5 h
Willow Flycatcher	*Empidonax traillii*	0.27
Willow/Alder Flycatcher	*Empidonax traillii/E. alnorum*	0.27
Northern Mockingbird	*Mimus polyglottos*	0.27
Green Heron	*Butorides virescens*	0.18
Gray Partridge	*Perdix perdix*	0.18
Belted Kingfisher	*Megaceryle alcyon*	0.18
Yellow-throated Vireo	*Vireo flavifrons*	0.18
Red-eyed Vireo	*Vireo olivaceus*	0.18
Tufted Titmouse	*Baeolophus bicolor*	0.18
White-breasted Nuthatch	*Sitta carolinensis*	0.18
Wood Thrush	*Hylocichla mustelina*	0.18
Northern Harrier	*Circus cyaneus*	0.09
Sora	*Porzana carolina*	0.09
Yellow-billed Cuckoo	*Coccyzus americanus*	0.09
Ruby-throated Hummingbird	*Archilochus colubris*	0.09
Eastern Wood-Pewee	*Contopus virens*	0.09
Eastern Phoebe	*Sayornis phoebe*	0.09
Loggerhead Shrike	*Lanius ludovicianus*	0.09
Yellow Warbler	*Dendroica petechia*	0.09
Yellow-headed Blackbird	*Xanthocephalus xanthocephalus*	0.09

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Records of Nine-Banded Armadillos, *Dasypus novemcinctus*, in Illinois

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ABSTRACT

The nine-banded armadillo, *Dasypus novemcinctus*, has been expanding its range in the United State since the mid-1800s. There had been sporadic reports of this species in Illinois prior to 1990. I conducted a mail survey in 2003 of individuals knowledgeable about the fauna of southern Illinois to investigate the possible expansion of the armadillo into the state. I also sent questionnaires to animal control agencies of 22 southern Illinois counties and municipalities. The questionnaire asked about armadillo sightings in Illinois since 1990. I mailed 158 questionnaires and received 101 responses. Twenty-three respondents knew of recent armadillo sightings and ten had observed armadillos personally. I collected additional records from a variety of sources, including biologists, members of the public, and scientific collections. From 1990 through March 2008 at least 166 armadillos were documented or reported from 42 Illinois counties. Some live animals were observed, but most reports were of apparent roadkills. Ninety-one percent of the sightings occurred after 2000. Eighty-eight percent of armadillo locations that could be mapped were south of a line through central Calhoun and southern Greene counties (approximately 39°N latitude).

INTRODUCTION

The nine-banded armadillo, *Dasypus novemcinctus*, is the only species in the Neotropical family Dasypodidae (order Cingulata) that occurs in the United States (Layne 2003). The species was first documented in the United States in southern Texas in the mid-1800s (Audubon and Bachman 1854). Since then its range has expanded dramatically, through natural dispersal and human intervention (Buchanan and Talmage 1954, Humphrey 1974, McBee and Baker 1982, Taulman and Robbins 1996, Freeman and Genoways 1998, Layne 2003). The species' northward expansion is limited by severity of winter weather because it is unable to hibernate (Humphrey 1974, Jones et al. 1983, Taulman and Robbins 1996), but its potential range might include the southern part of Illinois (Taulman and Robbins 1996). There have been occasional reports of armadillos in the state (e.g. Schwegman 1992, Taulman and Robbins 1996) and two specimens collected in Illinois as of 2000 are in mammal collections. The Illinois State University collection includes a roadkilled armadillo found in Empire Township, McLean County in 1999 (ISU 1215). The Illinois Natural History Survey (INHS) has part of the carapace of a roadkilled armadillo collected near Boody, Macon County in 2000 (INHS 818; Van Deelen et al. 2002).

Recent sightings known to Illinois Department of Natural Resources biologists prompted an investigation of the possible expansion of the range of the nine-banded armadillo into Illinois. I conducted a mail survey of people knowledgeable about the fauna of southern Illinois in 2003. Subsequently I collected additional records from a variety of sources. This paper presents information on armadillo records in Illinois as of 31 March 2008.

RANGE EXPANSION OF THE NINE-BANDED ARMADILLO

Dasypus novemcinctus has the most extensive geographical range of any armadillo species, extending from Argentina and Uruguay through Central America and most of Mexico (McBee and Baker 1982). Audubon and Bachman (1854) first documented the species in the United States in 1849, noting its presence in the lower Rio Grande Valley of southern Texas. It was largely restricted to the Rio Grande Valley until the early 1870s (Strecker 1926). In 1905 armadillos were common in southern Texas (Bailey 1905) and occasional individuals may have occurred in extreme southeastern New Mexico (Bailey 1931). By 1925 the armadillo was common in central Texas and had appeared in northwestern Louisiana (Strecker 1926). It soon spread into northeastern Louisiana (Strecker 1928) and was recorded east of the Mississippi River in Louisiana in 1935 (Lowery 1936). Armadillos were reported in Arkansas in 1921 (Dellinger and Black 1940) and first recorded in northeastern Oklahoma in 1932 (Blair 1936).

In the early 20th century the nine-banded armadillo was introduced into Florida at two locations (Bailey 1924, Sherman 1936). The species spread rapidly along the central east coast during the late 1930s and 1940s (Fitch et al. 1952). An isolated population that had become established in southern Alabama by 1949 presumably represented descendents of introduced animals (Fitch et al. 1952, Buchanan and Talmage 1954). The armadillo's range in 1954 (Figure 1a) included all or parts of Texas, Oklahoma, Arkansas, Louisiana, and peninsular Florida (Buchanan and Talmage 1954). Buchanan (1958) confirmed that the species also was established in southwestern Mississippi. By 1950 there were extralimital records (pioneers or introduced individuals) in Kansas (Hibbard 1943), Missouri (Anonymous 1947), and Georgia (Fitch et al. 1952).

As of 1972 the permanent range of the nine-banded armadillo (Figure 1b) covered all or parts of Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Alabama, Florida, and Georgia (Humphrey 1974). There were extralimital records for Tennessee (Humphrey 1974), Colorado (Hahn 1966), and South Carolina (Humphrey 1974) as well as more records in Kansas (e.g. Getz 1961, Smith and Lawlor 1964). By 1995 (Figure 1c) the armadillo had increased its range in Texas, Oklahoma, Arkansas, Alabama, Mississippi, Georgia, and Florida and also had become established in parts of Kansas, Missouri, Tennessee, and South Carolina (Taulman and Robbins 1996). Extralimital records existed as far north as Nebraska (Taulman and Robbins 1996, Freeman and Genoways 1998).

Thus, the nine-banded armadillo's expansion northward and eastward from Texas (and in all possible directions from Florida and Alabama) has been well documented. Humphrey (1974) determined that average invasion rates for the species were 4-10 km/yr; these rates are considered rapid for mammals (Taulman and Robbins 1996). Over the period 1972-1995, expansion of the Texas population to the northeast occurred at a rate of 11.2 km/yr,

expansion through Oklahoma into Kansas at about 11 km/yr, and expansion north from the Gulf Coast at 17.2 km/yr (Taulman and Robbins 1996).

METHODS

In 2003 I mailed a questionnaire to 136 individuals considered knowledgeable about the fauna of southern Illinois. The recipients were Illinois Department of Natural Resources (IDNR) District Wildlife Biologists (14), District Foresters (9), and Natural Heritage Biologists (7); Illinois Nature Preserves Commission field staff (4); IDNR biologists at Prairie Ridge and Cache River state natural areas (2); Conservation Police Officers in IDNR Regions IV and V (48); superintendents of state parks, fish and wildlife areas, conservation areas, and other state properties (37); Shawnee National Forest biologists (2); National Wildlife Refuge (NWR) managers (3); The Nature Conservancy biologist at Cypress Creek NWR (1); the U.S. Army Corps of Engineers superintendent at Carlyle Lake (1); and academics (8). I also mailed questionnaires addressed to the person responsible for animal control in 22 southern Illinois counties and municipalities.

The questionnaire asked if the recipient had personally observed nine-banded armadillos in Illinois since 1990 and, if so, when and where the animals had been seen and whether they were dead or alive. It also asked for details of other recent armadillo sightings that the recipient considered reliable. In a cover letter I requested that information on future sightings be sent to me.

I also posted a request for armadillo sightings on IBET, an internet listserve for Illinois birders in 2003. I notified the Bureau of Design and Environment (BDE) of the Illinois Department of Transportation that I was interested in observations of armadillo roadkills. Although unable to collect such information systematically, BDE staff forwarded some sightings by highway workers in southern Illinois. In addition, I contacted Dr. Russell Graham who, while at the Illinois State Museum in 1993, asked Illinois and Missouri residents to report armadillo sightings (Graham 1993).

In 2005 and again in 2008 I checked the mammal collections of the Field Museum of Natural History, Illinois State Museum, University of Illinois Museum of Natural History, Western Illinois University, Eastern Illinois University, Southern Illinois University, Cooperative Wildlife Research Laboratory (Southern Illinois University), and Southern Illinois University-Edwardsville for nine-banded armadillo specimens collected in Illinois. I searched MaNIS (http://manisnet.org) in 2008 for specimens in mammal collections outside Illinois.

Publicity about the armadillo survey (e.g. Hofmann and Esker 2005, Hofmann 2007, newspaper articles) subsequently led to communications from biologists and members of the public about additional armadillo sightings.

RESULTS

I received 101 responses from the 158 questionnaires that were mailed (63.9%). Twenty-three of the respondents (22.8%) knew of at least one armadillo sighting in Illinois since 1990. Two of these respondents did not describe specific sightings, but referred me to

other IDNR personnel who had already received questionnaires. Ten respondents (9.9%) had observed armadillos personally. Seven of these reported a single armadillo, two had seen two animals, and one (Lloyd Nelson, animal control officer for Jackson County) reported multiple records for 2000, 2001, and 2002.

As of 31 March 2008 I had received approximately 90 telephone calls or e-mails about armadillo sightings in Illinois. There was documentation of several of these armadillos; 18 were photographed (I took two photos and have seen eight others) and 13 specimens were collected by or given to IDNR personnel. Physical vouchers are being retained for the INHS mammal collection and five carcasses have been necropsied at the University of Illinois School of Veterinary Medicine Diagnostic Laboratory. I considered nearly all reports valid because the uniqueness of the armadillo makes it unlikely to be confused with any other Illinois mammal. It is possible, though unlikely, that an armadillo was a different species than *Dasypus novemcinctus*.

Sometimes it was clear that more than one person had reported the same armadillo. In a few cases it was not possible to ascertain if separate reports referred to the same animal. The exact number of individual armadillos reported is thus uncertain. From all sources, I collected observations of 161-167 individuals in Illinois from 1990 through March 2008. According to survey respondents, additional armadillos reportedly had been seen in Alexander and Pope counties and there may have been other armadillo sightings of which I am unaware. I found no specimens of free-living armadillos from Illinois that had been added to scientific collections since 2000 (other than vouchers retained at the INHS). Single specimens from McLean (ISU 1215) and Macon (INHS 818) counties were collected (in 1999 and 2000, respectively) and three additional armadillos were documented after 1990 (Schwegman 1992, Taulman and Robbins 1996). Thus, at least 166 armadillos have been reported in Illinois since 1990.

Most of the armadillos reported in Illinois were dead; these were typically found along roads and presumably killed by vehicles. Thirty-one live animals were reported, although at least four of these were known to have been killed subsequently (by vehicles, a gunshot, or a dog). Observations of live armadillos were reported for each year from 2001 through 2008 and some were seen during winter months. People were uncertain about the year of some early armadillo observations, but very few sightings from the 1990s were reported. The vast majority (91%) of sightings occurred from 2001 through March 2008.

I collected records of armadillos from 40 counties for 1990 through March 2008 (Figure 2). Two armadillos were observed near the White/Edwards and Moultrie/Shelby county lines, but only one county for each sighting was included in Figure 2. The specimens from McLean and Macon counties were collected during this period, bringing the total number of Illinois counties with armadillo reports since 1990 to 42. The largest number of armadillo reports (44-48) was from Jackson County, partly because the county animal control officer had kept records of armadillo sightings. Other counties with relatively large numbers of reported observations were Randolph (16-17), St. Clair (10), Clinton (8), and Monroe (7-9). I received only one or two specific armadillo reports for 22 counties (Alexander, Christian, Clay, Cook, DuPage, Edgar, Effingham, Fayette, Fulton, Hardin, Henry, Marion, Montgomery, Moultrie, Perry, Pike, Pope, Pulaski, Scott, Tazewell, Washington, and White).

Figure 3 shows locations of 114 armadillos reported during this survey. Other reports, including many provided by Jackson County Animal Control, were not specific enough to map the animal's location. Also included in Figure 3 are localities for the specimens from McLean and Macon County and an armadillo found in Pope County in 1991 (Schwegman 1992). Although a few armadillos were observed or collected in northern and central Illinois, most have been reported in the southern half of the state. Eighty-eight percent of the armadillos whose locations are shown in Figure 3 (as well as almost all animals that could not be mapped) occurred south of a line through central Calhoun and southern Greene counties. Calhoun County locations were clustered near Hardin, which is at 39°09'N latitude. Ninety percent of the southern Illinois armadillo locations in Figure 3 were along or west of the I-24/I-57 highway corridor (i.e. in the western portion of southern Illinois).

Climatological Data

The nine-banded armadillo has become established on the Great Plains in areas where mean temperatures for January (typically the coldest month) are between 0°C and -2.0°C (Taulman and Robbins 1995). For comparison, I examined winter temperatures along a latitudinal gradient in southern Illinois. Climatological data from five weather stations (Cairo, Carbondale, Belleville, Alton, and Jerseyville) for January 1995-March 2008 were obtained from the Illinois State Water Survey (www.sws.uiuc.edu/data/climatedb). Table 1 shows January temperature information for these stations. Mean January temperature at Cairo in the southern tip of Illinois (Alexander County; 37°00'N latitude) averaged 2.0°C for 1995 through 2008. The January mean was below 0°C twice, but never below -2.0°C (the lowest January mean being -1.7°C in 2003). For the winter of 2000-2001 December was the coldest month at Cairo; it was the only month during 14 winters with a mean temperature less than -2.0°C (-3.4°C). Mean January temperatures at Carbondale (Jackson County; 37°43'N) averaged 0.9°C for 1995-2008. The lowest January mean during this period was -2.5°C in 2003. December 2000 was the only other month during 14 winters with a mean temperature below -2.0°C (-4.8°C) in Carbondale. In Belleville (St. Clair County; 38°31'N) mean January temperatures for 1995-2008 averaged 1.4°C and never were below -2.0°C. As with Cairo, December 2000 was the only month that had a mean temperature less than -2.0°C.

Temperature data for January for Alton (Madison County; 38°53'N) only were available for 11 years between 1995 and 2008. Mean January temperatures averaged -0.4°C and were below -2.0°C for three of 11 winters. December 2000 again was the coldest month, with a mean temperature of -5.4°C. At Jerseyville (Jersey County; 39°07'N) mean January temperatures were available for 12 years between 1995 and 2008 and averaged -1.7°C. The January mean was below -2.0°C during six of 12 winters; the lowest January mean was -5.4°C in 1997. Three additional months had mean temperatures less than -2.0°C. For the period 2000-2008, when most of the armadillos were reported in Illinois, averages of mean January temperatures for these five weather stations were 0.1 to 1.1°C higher.

Table 1. January temperature information for five weather stations in southern Illinois, 1995-2008 (Illinois State Water Survey, www.sws.uiuc.edu/data/climatedb). Data were not available for all 14 years at Alton and Jerseyville. Because sample sizes were small, only averages of mean January temperatures are shown. The numbers of years during which the mean January temperature was lower than 0°C and -2°C also are presented.

station	average Jan. mean (°C)	# years < 0°C	# years < -2°C
Cairo	2.0	2/14	0/14
Carbondale	0.9	5/14	1/14
Belleville	1.4	4/14	0/14
Alton	-0.4	6/11	3/11
Jerseyville	-1.7	9/12	6/12

DISCUSSION

Only sporadic occurrences of nine-banded armadillos had been reported in Illinois prior to 1990. Schwegman (1992) recalled a newspaper account of an armadillo seen in Union County in the late 1970s and provided a report of a roadkilled animal in Hardin County in the 1980s. The Cooperative Wildlife Research Laboratory performed a necropsy on a moribund armadillo (CW84-7) found in Alexander County in December 1983 (A. Woolf, personal communication, 20 October 2003). A Conservation Police Officer reported a roadkilled animal in southern Illinois around 1990 (R. Graham, personal communication, 26 July 2004) and Schwegman (1992) described an armadillo in Pope County in 1991. Taulman and Robbins (1996) mapped two Illinois armadillos; these were 1994 roadkills from Union County (J. Taulman, personal communication, 27 May 2005). The first Illinois specimens were collected in 1999 (ISU 1215; A. Capparella, Illinois State University, personal communication, 28 January 2005) and 2000 (INHS 818; Van Deelen et al. 2002).

I collected reports of six additional armadillo sightings in Illinois that definitely occurred during the 1990s, but there have been reports of at least 147 sightings since 2000. These findings raise two basic questions. First, how did these animals get to Illinois? Second, has the species become established in Illinois, i.e. is a breeding population present or is there a continual influx of animals from elsewhere?

Natural range expansion involves movement of individuals outward from the edge of a species' range. There is no doubt, however, that human translocation has played a major role in the armadillo's spread (e.g. Fitch et al. 1952, Buchanan and Talmage 1954, Jones et al. 1983, Taulman and Robbins 1996). Armadillos captured as pets or curiosities can later be released or escape long distances from their original locations. Release or escape of individuals led to the establishment of armadillo populations in Florida and Alabama (Fitch et al. 1952, Buchanan and Talmage 1954). In addition, there can be translocation of armadillos into new areas as "hitchhikers" in trucks, railroad cars, and barges. Fitch et

al. (1952), for example, recounted that live armadillos frequently escaped from boxcars when cattle trains from Texas arrived in Rankin County, Mississippi during the 1930s.

The nine-banded armadillo occupies Missouri as far north as the Missouri River (Robbins et al. 1994), but armadillos are found only occasionally in Kentucky (http://fw.ky.gov/kfwis/speciesinfo/speciesinfo.asp). Thus, Illinois is separated from the nearest population from which natural dispersal could occur by the Mississippi River. Armadillos can swim (e.g. Taber 1945), but it is not known if they are able to swim far enough to cross the Mississippi River at southern Illinois. "Island-hopping" may enable armadillos to cross it in stages. It also might be possible that they use highway bridges, but I received no reports of roadkilled armadillos on bridges over the Mississippi River. Armadillos could enter Illinois from other states on trucks, trains, or the barges that travel up the Mississippi, Ohio, and Illinois Rivers. Finally, people could intentionally transport armadillos into Illinois and release them (as pranks or after having second thoughts about keeping them as pets). Although the way in which armadillos arrived in Illinois cannot be determined, it is likely that people translocated animals intentionally or unintentionally. This is especially likely for armadillos observed or collected in the northern half of the state.

There is consensus that climate will limit the distribution of the nine-banded armadillo (e.g. Taber 1939, Humphrey 1974, Jones et al. 1983, Taulman and Robbins 1996). Because armadillos have high thermal conductance (McNab 1980) and cannot hibernate, their northward expansion will be limited by the severity of winter temperatures. Since these temperatures vary from year to year, the northern edge of the armadillo's distribution will be fluid. For example, there were fewer armadillo sightings in northern Kansas in 2001, following the very cold winter of 2000-2001 (Merriam 2002). Taber (1939) thought that cold would prevent the armadillo's establishment north of 33°N. By 1954, however, the species was established close to 35°N (Buchanan and Talmage 1954), while Humphrey (1974) suggested that the "winter barrier" might be moving northward. The armadillo's range in 1995 reached about 38°N latitude in Kansas (Taulman and Robbins 1996). Taulman and Robbins (1996) found that the species was established in areas on the Great Plains with mean January temperatures between 0°C and -2.0°C. These regions tend to have fewer than 24 freeze-days annually, although the number of consecutive freeze-days may be more limiting than the total number (Taulman and Robbins 1996). Taulman and Robbins (1996) produced an isotherm for a mean January temperature of -2.0°C, based on climate data through 1990, which crossed Illinois at about 39°N latitude (north of Alton).

Although there was a cluster of armadillo reports in Jersey, Greene, and Calhoun counties (Figure 3), temperature records since 1995 for Jerseyville (where the mean January temperature averaged -1.7°C) suggest that the species might survive that far north only during mild winters. Mean monthly temperatures were only infrequently less than -2.0°C at the Belleville, Carbondale, and Cairo weather stations. Thus, winter temperatures in Illinois south of the East St. Louis metro region should be suitable for the establishment of nine-banded armadillo populations. Depth and duration of snow cover or prolonged periods of below-freezing temperatures, however, also would likely affect armadillo survival. Even in far southern Illinois mortality could increase during harsh winters, reducing armadillo numbers or even eliminating them from the state. Given the potential for inten-

tional and unintentional translocation of armadillos, they should be able to repopulate southern Illinois under favorable climatic conditions.

It has not yet been demonstrated that a breeding population of nine-banded armadillos has become established in Illinois. Necropsies revealed that two females found in the state contained fully formed fetuses. Superdelayed parturition has been documented in this species, with gestation lasting as long as 24 months (Storrs et al. 1988). Therefore, it is possible that these females mated elsewhere and subsequently dispersed or were transported into Illinois. The large number of sightings in recent years and the fact that live animals have been seen during winter, however, suggest the possibility that some armadillos are surviving and breeding in the southern part of the state. Humphrey (1974) felt that range maps should make a distinction between areas with permanent armadillo populations and those where only pioneering individuals occurred. Genoways et al. (2000:93) defined the pioneering zone of a species as "the area where, under favorable conditions, the species is capable of reproducing and conducting its normal activities." At the present time Illinois can be considered, at least, part of the pioneering zone of the nine-banded armadillo.

SUMMARY

At least 166 nine-banded armadillos have been documented or reported in Illinois from 1990 through March 2008. The vast majority of these animals (91%) have been observed since 2001. Armadillos have been reported or collected in 42 counties. Most mapped locations of armadillos (88%) were south of a line through central Calhoun and southern Greene counties (approximately 39°N latitude). It is likely that people intentionally or unintentionally transported these armadillos into the state. The relatively mild winters in the southern third of Illinois should be favorable for armadillos to survive and reproduce, but the establishment of a breeding population in Illinois has not yet been demonstrated.

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LITERATURE CITED

Anonymous. 1947. A visitor from the south. Missouri Conservationist 8(5):12.

Audubon, J. and J. Bachman. 1854. The quadrupeds of North America. Volume 3. V.G. Audubon, New York, NY. 348 p.

Bailey, H.H. 1924. The armadillo in Florida and how it reached there. Journal of Mammalogy 5:264-265.

Bailey, V. 1905. Biological survey of Texas. North American Fauna 25:1-222.

Bailey, V. 1931. Mammals of New Mexico. North American Fauna 53:1-412.

Blair, W.F. 1936. The nine-banded armadillo in northeastern Oklahoma. Journal of Mammalogy 17:292-294.

Buchanan, G.D. 1958. The current range of the armadillo *Dasypus novemcinctus mexicanus* in the United States. Texas Journal of Science 10:349-351.

Buchanan, G.D. and R.V. Talmage. 1954. The geographical distribution of the armadillo in the United States. Texas Journal of Science 6:142-150.

Dellinger, S.C. and J.D. Black. 1940. Notes on Arkansas mammals. Journal of Mammalogy 21:187-191.

Fitch, H.S., P. Goodrum, and C. Newman. 1952. The armadillo in the southeastern United States. Journal of Mammalogy 33:21-37.

Freeman, P.W. and H.H. Genoways. 1998. Recent northern records of the nine-banded armadillo (*Dasypus novemcinctus*) in Nebraska. Southwestern Naturalist 43:491-504.

Genoways, H.H., P.W. Freeman, and C. Grell. 2000. Extralimital records of the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*) in the central United States and their biological significance. Transactions of the Nebraska Academy of Sciences 26:85-96.

Getz, L.L. 1961. New locality records of some Kansas mammals. Journal of Mammalogy 42:282-283.

Graham, R.W. 1993. Armadillos: invasion or return? The Living Museum 55(1):3-4.

Hahn, D.E. 1966. The nine-banded armadillo, *Dasypus novemcinctus*, in Colorado. Southwestern Naturalist 11:303.

Hibbard, C.W. 1943. A checklist of Kansas mammals. Transactions of the Kansas Academy of Science 47:61-88.

Hofmann, J. 2007. Armadillo odyssey. OutdoorIllinois 15(3):16-17.

Hofmann, J. and T. Esker. 2005. Was that an armadillo I just saw? Illinois Natural History Survey Reports 382:1&8.

Humphrey, S.R. 1974. Zoogeography of the nine-banded armadillo *(Dasypus novemcinctus)* in the United States. BioScience 24:457-462.

Jones, J.K., Jr., D.M. Armstrong, R.S. Hoffmann, and C. Jones. 1983. Mammals of the northern Great Plains. University of Nebraska Press, Lincoln. 379 p.

Layne, J.N. 2003. Armadillo: *Dasypus novemcinctus*. Pages 75-97 in Wild mammals of North America: biology, management, and conservation. G.A. Feldhamer, B.C. Thompson, and J.A. Chapman (eds). Second edition. The Johns Hopkins University Press, Baltimore, MD.

Lowery, G.H., Jr. 1936. A preliminary report on the distribution of the mammals of Louisiana. Proceedings of the Louisiana Academy of Sciences 3:11-39.

McBee, K. and R.J. Baker. 1982. *Dasypus novemcinctus*. Mammalian Species 162:1-9.

McNab, B.K. 1980. Energetics and the limits to a temperate distribution in armadillos. Journal of Mammalogy 61:606-627.

Merriam, D.F. 2002. The armadillo [*Dasypus novemcinctus* (Linnaeus)] invasion of Kansas. Transactions of the Kansas Academy of Science 105:44-50.

Robbins, L.W., K.J. Lippert, and P.T. Schell. 1994. The ecology and impact of the armadillo (*Dasypus novemcinctus*) in Missouri. Unpublished report to the Missouri Department of Conservation. Southwest Missouri State University, Springfield. 32 p.

Schwegman, J. 1992. The armadillos are coming. Illinois Department of Conservation news release, 11 August 1992.

Sherman, H.B. 1936. List of the recent wild land mammals of Florida. Proceedings of the Florida Academy of Science 1:102-128.

Smith, J.D. and T.E. Lawlor. 1964. Additional records of the armadillo in Kansas. Southwestern Naturalist 9:48-49.

Storrs, E.E., H.P. Burchfield, and J.W. Rees. 1988. Superdelayed parturition in armadillos: a new mammalian survival strategy. Leprosy Review 59:11-15.

Strecker, J.K. 1926. The extension of the range of the nine-banded armadillo. Journal of Mammalogy 7:206-214.

Strecker, J.K. 1928. The nine-banded armadillo in northeastern Louisiana. Journal of Mammalogy 9:69-70.

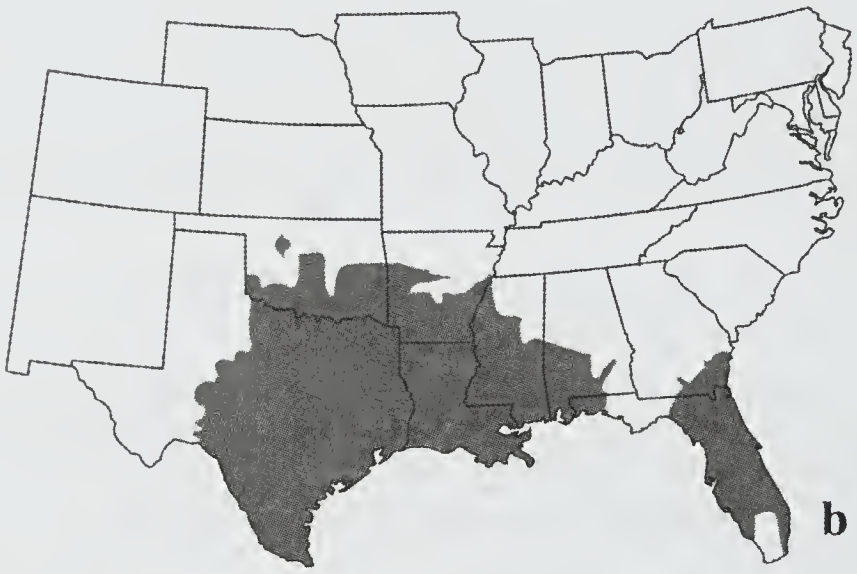
Taber, F.W. 1939. Extension of the range of the armadillo. Journal of Mammalogy 20:489-493.

Taber, F.W. 1945. Contribution on the life history and ecology of the nine-banded armadillo. Journal of Mammalogy 26:211-226.

Taulman, J.F. and L.W. Robbins. 1996. Recent range expansion and distributional limits of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States. Journal of Biogeography 23:635-648.

Van Deelen, T.R., J.D. Parrish, and E.J. Heske. 2002. A nine-banded armadillo (*Dasypus novemcinctus*) from central Illinois. Southwestern Naturalist 47:489-491.

Figure 1. Distribution maps for the nine-banded armadillo in the United States: (a) range in 1954, after Buchanan and Talmage 1954; (b) range in 1972, after Humphrey 1974; (c) range in 1995, after Taulman and Robbins 1996.



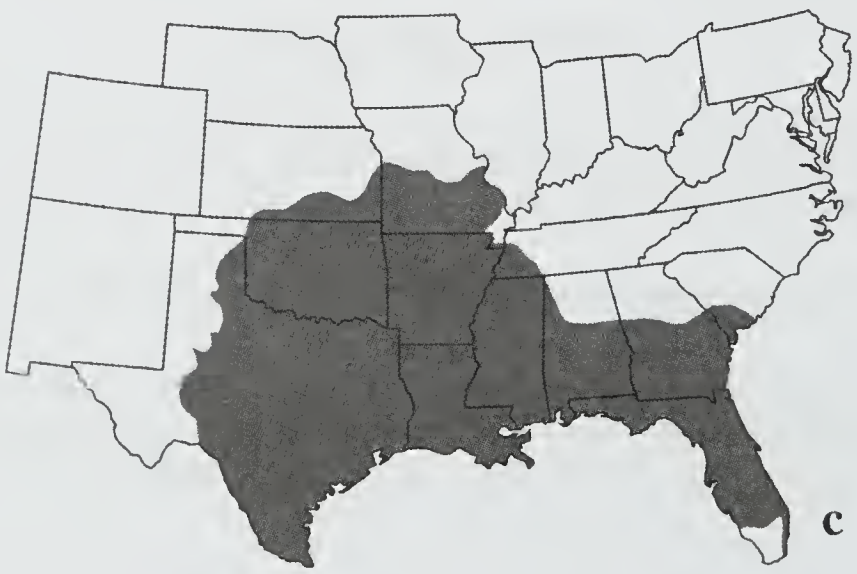


Figure 2. Illinois counties with nine-banded armadillos sightings, 1990-March 2008. Solid fill = counties with reports collected during the present study, cross-hatching = counties represented by museum specimens (ISU 1215 and INHS 818).

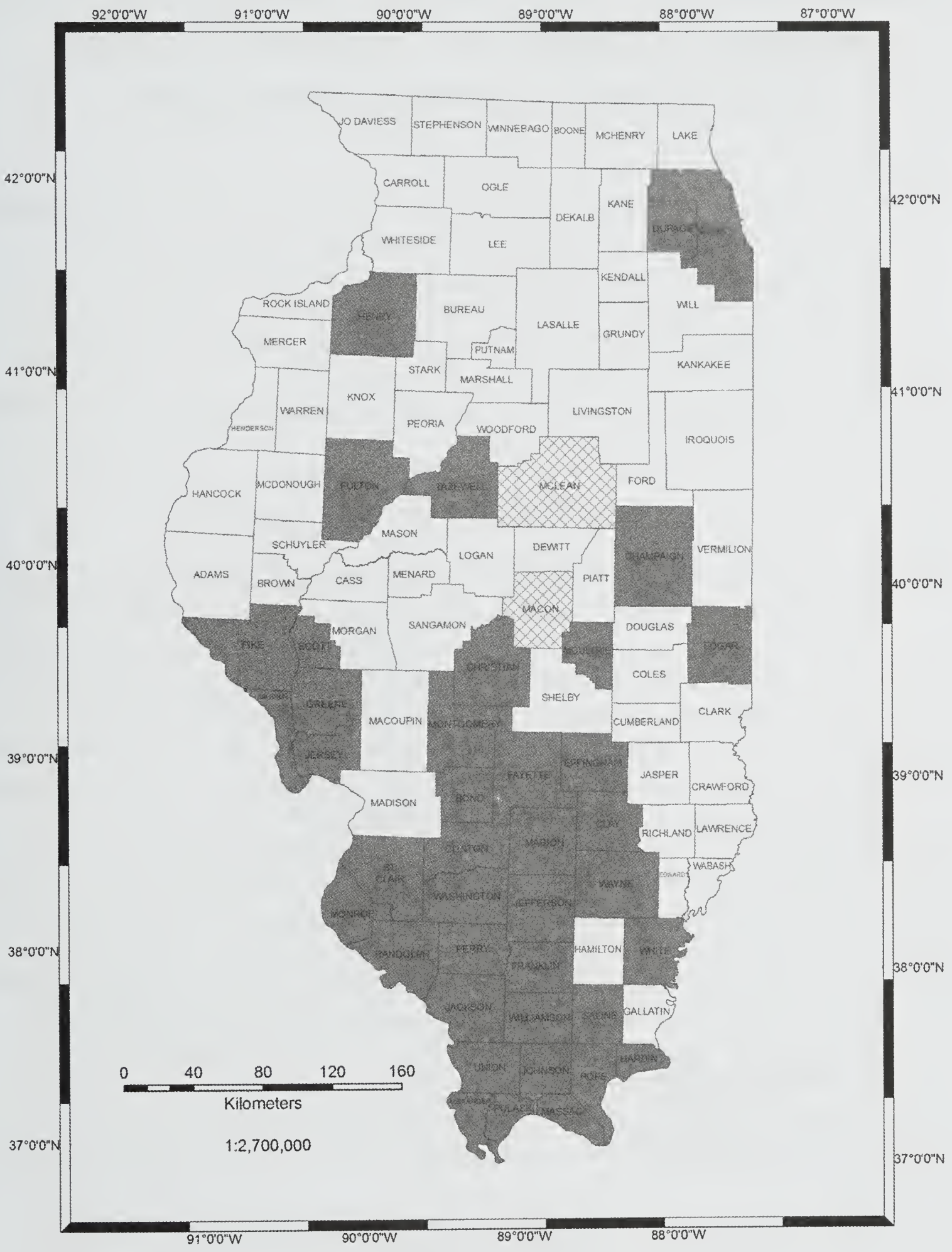
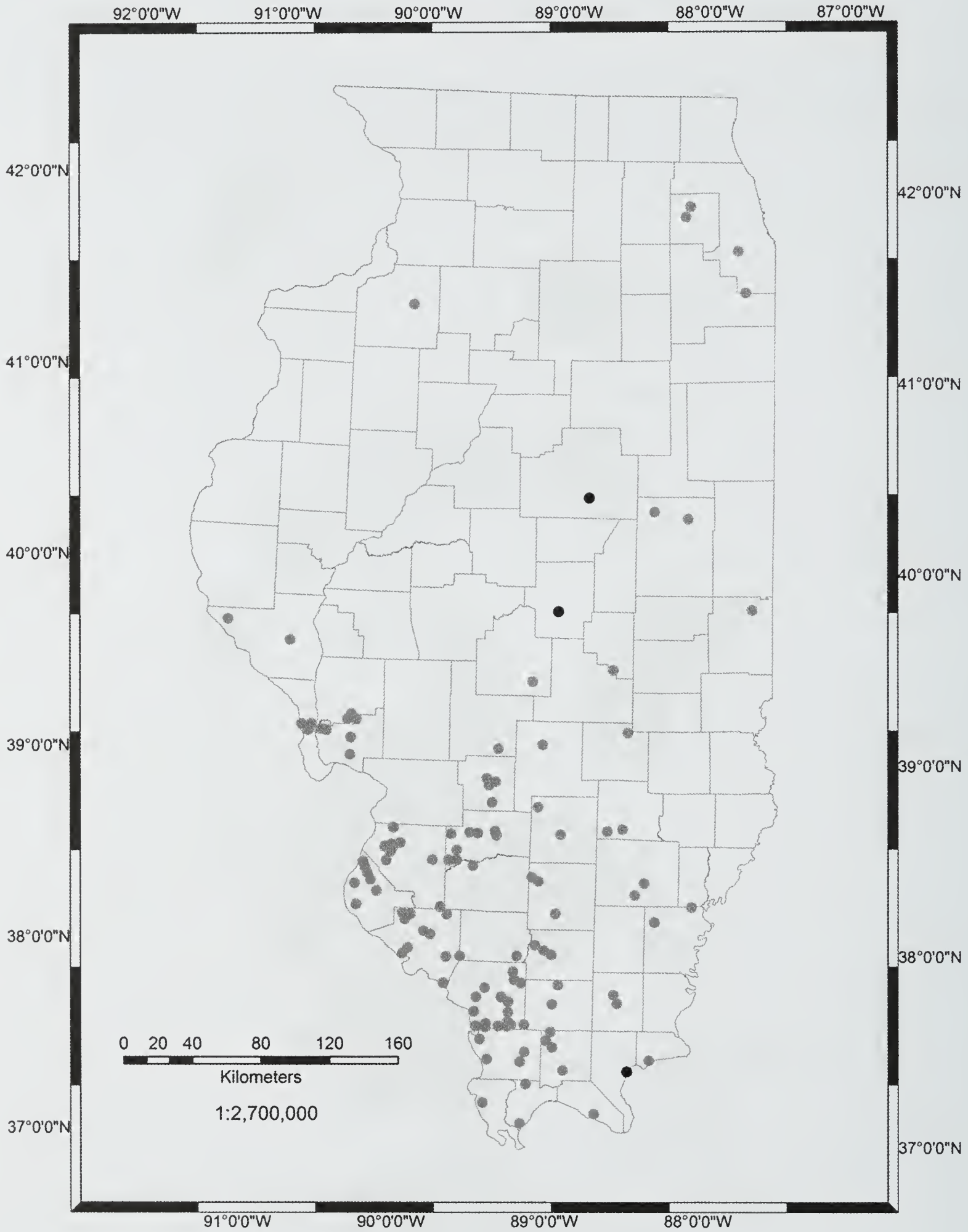


Figure 3. Locations of nine-banded armadillo sightings in Illinois, 1990 – March 2008. Gray dots = sightings collected during the present study, black dots = specimens (ISU 215 and INHS 818) and Pope County location from Schwegman (1992).



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Abundance and Diversity of Thread-Waisted Wasps (Hymenoptera: Sphecidae: Sphecinae) at Alice L. Kibbe Life Science Station, Hancock County, Illinois USA

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ABSTRACT

Sphecid wasps (Hymenoptera: Sphecidae) represent a diverse group of predatory wasps that includes potentially important bioindicators and biological control agents. Thread-waisted wasps (Hymenoptera: Sphecidae: Sphecinae) were surveyed in four habitats (recently burned prairie – burned in spring 2005, previously burned prairie – last burned in spring 2004, recently burned oak-hickory forest – burned in spring 2005, and unburned oak-hickory forest – not burned for 5+ years) in west-central Illinois from May through October 2005 using malaise traps. Ten species, representing six genera, were collected. *Ammophila nigricans* Dahlbom, *Eremnophila aureonotata* (Cameron), and *Sphex nudus* Fernald were the most abundant species, representing over 90% of total captures, with *A. nigricans* alone accounting for almost 75% of all captures. Recently burned prairie produced the greatest overall abundance and species richness, and forest habitats produced the lowest. Relative proportions of sphecine captures in recently burned vs. previously burned prairie decreased late in the season. Greatest numbers of sphecines were captured in July. Seasonal activity patterns were generally similar to those of sphecines further north, but may extend somewhat longer in west-central Illinois for some species. Our results suggest that sphecines are most abundant and active in prairie habitats at our study site, and that three species dominate the sphecine fauna there. Fire and subsequent recovery of prairie may have short-term effects on sphecine abundance and activity patterns.

INTRODUCTION

The Sphecinae (Hymenoptera: Sphecidae) is a diverse subfamily of large, often brightly colored wasps commonly known as "thread-waisted wasps" because of the distinctive slender petiole of the metasoma. The subfamily consists of 11 genera and 127 species in North America (O'Brien, 1989). Most sphecine genera nest in cavities in the ground,

although some nest aerially and a few are inquilines (Bohart and Menke, 1963). Sphecines are predatory, provisioning the nest with arthropod prey. Sphecines are often prey specialists, with a genus specializing on a particular taxon, size, location (e.g., fossorial vs. arboreal caterpillars), or stage of prey (O'Brien, 1989). While sphecines are generally considered solitary, some display a primitive form of sociality (Bohart and Menke, 1976).

Spheciform wasps have received attention as potential biodiversity indicators and bioindicators of habitat quality and ecological disturbance (Tscharntke et al., 1998; Gayubo et al., 2005), and wasp species richness has been shown to correlate with landscape complexity and habitat diversity (Steffan-Dewenter, 2002). Sphecid wasps can also be important as biological control agents (Frank et al., 1995).

Western Illinois University's Alice L. Kibbe Life Science Station contains a mosaic of forest and restored prairie habitats that are being subjected to long-term management using controlled burning to suppress early successional and non-native plant growth. This landscape heterogeneity provides an excellent opportunity to examine the diversity of west-central Illinois sphecines in relation to habitat diversity and disturbance. The goals of our study were to: 1) characterize the sphecine fauna of prairie and forest habitats at Alice L. Kibbe Life Science Station, 2) examine seasonal activity patterns of the sphecine fauna, and 3) examine variation in sphecine abundance and diversity in relation to habitat.

MATERIALS AND METHODS

Sampling occurred May through October 2005 at Alice L. Kibbe Life Science Station near Warsaw in Hancock Co., Illinois USA. Habitats sampled included a restored prairie burned in spring 2005 (40° 22.0' N, 91° 24.3' W), a restored prairie last burned in spring 2004 (40° 21.8' N, 91° 24.3' W), an oak-hickory forest stand burned in spring 2005 (40° 22.0' N, 91° 24.5' W), and an oak-hickory forest stand unburned for 5+ years (40° 21.8' N, 91° 24.1' W). These habitats will be referred to as recently burned prairie, previously burned prairie, recently burned forest, and unburned forest, respectively. The prairie habitats were former agricultural fields that were restored to prairie in the late 1970s. The recently burned prairie was ca. 3.0 ha and linear, with a length of ca. 500 m and width of ca. 60 m. The previously burned prairie was irregular in shape and ca. 2.5 ha. Abundant plants in the prairie habitats included warm season bunch grasses such as Indian grass, *Sorghastrum nutans* (L.) Nash, big bluestem, *Andropogon gerardii* Vitman, little bluestem, *Schizachyrium scoparium* (Michx.) Nash, and switchgrass, *Panicum virgatum* L., as well as many forb species from the families Asteraceae and Fabaceae. Woody species included rough-leaved dogwood, *Cornus drummondii* C. A. Mey, and smooth sumac, *Rhus glabra* L. (Willand, 2006). Mean percent ground cover (± SE), as measured by visual estimation of 20 0.5-m^2 plots in mid-July 2005, was 55.5 ± 3.73 and 80.5 ± 3.80 in the recently burned prairie and previously burned prairie, respectively. In mid-Sept 2005, these measures were 71.0 ± 2.98 and 76.5 ± 2.54, respectively.

The forest habitats were old growth, dry-mesic upland oak-hickory forests. These stands were open woodland-savanna with a history of grazing until the 1950s-60s. Fire suppression allowed the emergence of a dense understory after this time. The recently burned forest had undergone prescribed burning every 2-3 years since the mid 1990s to suppress

understory vegetation, but the unburned forest had a history of more infrequent and inconsistent fire, and hadn't been burned in 5+ years (Willand, 2006). Total contiguous forest area was at least 200 ha, with burn units of ca. 3.0 ha each. Dominant overstory tree species included white oak, *Quercus alba* L., northern red oak, *Quercus rubra* L., and shagbark hickory, *Carya ovata* (Miller) K. Koch (Willand, 2006). Few invasive plant species were present. Mean percent ground cover (± SE) in the forest habitats was also measured by visual estimation of 20 0.5-m^2 plots. In mid-July 2005 these values were 29.0 ± 3.24 and 59.5 ± 3.36 in the recently burned forest and unburned forest, respectively. In mid-Sept 2005, these measures were 23.5 ± 2.84 and 38.0 ± 3.88, respectively. Plant nomenclature follows that of Gleason and Cronquist (1991).

Three malaise traps (Townes, 1972) were placed in each of the four habitats, for a total of 12 traps. Malaise traps are flight interception traps that are effective in capturing active flying insects such as Hymenoptera (Darling and Packer, 1988). Traps within a given habitat were placed a minimum of 75 m apart. Traps in prairie habitats were located 15 m to 30 m from the nearest forest/prairie interface, and traps in forest habitats were located 25 m to 50 m from the nearest forest/prairie interface. Trap collection bottles were filled with 70% EtOH. Traps were operated continuously from 16 May to 24 October. Samples were collected twice weekly, every three or four days, and the previous bottle was replaced with one containing fresh EtOH. Sphecine wasps were collected from the samples, pinned, and labeled with collection information. A representative sample of collected sphecines was identified by Dr. Wojciech Pulawski, of the California Academy of Science. We identified the remaining sphecines using this reference collection and Bohart and Menke (1963).

Sphecine species richness and Simpson's diversity indices (Simpson, 1949) were calculated for each habitat. Species richness is associated with sample size, so rarefaction was used in comparing species richness of different habitats. Rarefaction provides an estimate of the expected number of species for a given sample size (Krebs, 1999). The University of Alberta Department of Biology online rarefaction calculator (http://www.biology.ualberta.ca/jbrzusto/rarefact.php#Calculator) was used in these analyses. Simpson's index considers the number of species captured (species richness) but also the relative proportions of individuals that occur in each species. A collection of species that is dominated by one or a few species will have lower diversity than one in which individuals are apportioned equally among all species (Brower et al., 1998). Simpson's index ranges from 0 (low diversity) to near 1. Comparisons between Simpson's indices were done using *t*-tests following the methods of Keefe and Bergerson (1977). Relative abundances of sphecines in prairie habitats in relation to season (early vs. late) were analyzed using the *G*-test, with Yates' continuity correction.

RESULTS AND DISCUSSION

A total of 1981 sphecines (12.3/trap/day), representing 10 species and 6 genera, were collected over the course of the study (Table 1). Mean numbers of sphecines captured per trap (± SEM) were 472.3 ± 207.9 in the recently burned prairie (min = 222, max = 885), 175.3 ± 18.2 in the previously burned prairie (min = 144, max = 207), 10.0 ± 4.5 in the recently burned forest (min = 5, max = 19), and 2.67 ± 2.67 in the unburned forest (min = 0, max = 8). The first sphecine, *Chalybion californicum* (de Saussure), was collected on

30 May, and the last, *Ammophila urnaria* Dahlbom, on 10 October. Greatest numbers of sphecines were collected during July (1053 specimens), with a peak collection date of 11 July (263 specimens). Three species (*Ammophila nigricans* Dahlbom, *Eremnophila aureonotata* (Cameron), and *Sphex nudus* Fernald) comprised 91.5% of total captures, with *A. nigricans* alone accounting for almost 75% of all captures (Table 1).

Each sphecine species was more abundant in prairie than in forest habitats, with the exception of *Podium luctuosum* F. Smith (Table 1). This is an uncommonly collected species that inhabits mesic woodlands, constructing cells under bark flaps or in tree holes (Krombein, 1979; O'Brien, 1989). Cells are provisioned with cockroaches. *Parcoblatta uhleriana* (de Saussure) and *Parcoblatta virginica* (Brunner) have been recorded as prey (Pate, 1949; Krombein, 1967), and both, as well as several other species of *Parcoblatta*, occur in Illinois (Hebard, 1917). However, this species will move into more open habitats as evidenced by our capture of three specimens in two different traps in the recently burned prairie. Among species other than *P. luctuosum*, *E. aureonotata* produced the greatest proportion of captures in forest traps (7.1%), suggesting that this species may tend to range more widely into different habitats than other open-habitat sphecines. Among the remaining eight species, only 1.1% (19 of 1763 individuals) was collected in forest traps (Table 1).

Among prairie captures, greater numbers of each sphecine species were collected in the recently burned than in the previously burned prairie (Table 1).The recently burned prairie produced the greatest sphecine numbers and species richness, and both prairie habitats combined comprised 98.1% of all sphecines collected. Observed species richness was within one standard deviation of the expected richness based on rarefaction for all habitats except the previously burned prairie, which had substantially lower species richness than expected (Table 1). Diversity was significantly greater in recently burned than in previously burned prairie (0.47 vs. 0.30; $t = 5.714$, df $= \infty$, $P < 0.001$), but was lower overall in prairie than in forest habitats, due primarily to the dominance of *A. nigricans* in the prairie habitats (Table 1).

It is possible that the reduced vegetation and more open habitat produced by burning created more favorable conditions for sphecine prey location or nesting activities. Of the six genera collected in this study, three (*Ammophila*, *Eremnophila*, and *Sphex*) are fossorial nesters (O'Brien, 1989), and the three most abundant species were in these genera (Table 1). There was also a temporal pattern with regard to relative abundances of these three species in the two prairie habitats. For *A. nigricans*, 70.9% of early season (through July) captures occurred in the recently burned prairie, whereas only 34.0% of late season (after July) captures occurred in the recently burned prairie ($G = 26.46$, df $= 1$, $P < 0.0001$). Similar reductions in late season proportions of recently burned prairie captures occurred for *E. aureonotata* (from 96.6% to 52.5%; $G = 55.02$, df $= 1$, $P < 0.0001$) and *S. nudus* (from 98.6% to 76.1%; $G = 16.21$, df $= 1$, $P < 0.0001$). This relative decrease in proportion of recently burned prairie captures later in the season may reflect the recovery of the vegetation in this habitat from very sparse post-burn levels to a level similar to the previously burned prairie, with concomitant effects on wasp hunting, nesting, or flight behavior.

Collection dates for the seven most abundant species are shown in Figs. 1 and 2. Data are presented based on weekly rather than biweekly trapping totals for clarity. Collections of *E. aureonotata* occurred during a two-month period from 11 July to 12 Sept, with peak collections in mid- to late July and early August. This is similar to the seasonal activity patterns of this species in Michigan (O'Brien, 1989). *Sphex nudus* showed activity patterns similar to *E. aureonotata* in our study, with peak collections in late July and early August (Fig. 1). *Ammophila nigricans* was first collected on 6 June, with peak abundance on 11 July. The latest collection of this species occurred on 5 September (Fig. 1). This species was collected 11 days earlier and 8 days later in our study than in Michigan, where 49 specimens were collected from 17 June to 28 August (O'Brien, 1989). This potential difference in seasonal activity could reflect the more extended warm weather season in west-central Illinois or the much greater sample size of this species in our study. Like its congeneric *A. nigricans*, *A. urnaria* was first collected on 6 June, but its activity was more prolonged than that of *A. nigricans*, extending to 10 October (Fig. 2). This is consistent with activity patterns of these two species in Michigan, where *A. nigricans* was not collected beyond late August, but *A. urnaria* has been collected as late as early October (O'Brien, 1989). Again, our latest collection of this species was 9 days later than was found in Michigan (O'Brien, 1989). *Ammophila urnaria* is the most commonly collected species of the genus in Michigan and probably the most common *Ammophila* in North America (O'Brien, 1989), but it was much less common than *A. nigricans* in our study (Table 1). Although greater sample sizes would be needed for clarification, our collection data are consistent with O'Brien's (1989) suggestion that *A. urnaria* may be bivoltine (Fig. 2). The first collection of *C. californicum* occurred on 30 May, with greatest numbers in mid to late June (Fig. 2). This blue mud dauber uses abandoned nests of the black and yellow mud dauber *Sceliphron caementarium* (Drury) (Rau, 1928), which was not collected in our study. Perhaps this latter species is less abundant in the vicinity of our study site, or is less susceptible to capture in malaise traps. Both *Isodontia mexicana* (de Saussure) and *Isodontia apicalis* (F. Smith) were first collected on 6 June, but *I. apicalis* was not collected again until July, whereas *I. mexicana* was consistently collected throughout June and July (Fig. 2).

In summary, *A. nigricans*, *E. aureonotata*, and *S. nudus* were the most abundant species of sphecines collected in our study. Sphecine abundance and diversity varied widely within a relatively small geographic area. Disturbance by fire appeared to affect sphecine abundance, activity, and diversity, but these effects were short-lived. In general, sphecine seasonal activity patterns at our study site were similar to those found further north, but may extend somewhat longer for some species.

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LITERATURE CITED

Bohart, R. M., and A. S. Menke. 1963. A reclassification of the sphecinae, with a revision of the Nearctic species of the tribes Sceliphronini and Sphecini (Hymenoptera, Sphecidae). University of California Publications in Entomology 30: 91-181.

Bohart, R. M., and A. S. Menke. 1976. Sphecid Wasps of the World: a Generic Revision. University of California Press, Berkeley.

Brower, J. E., J. H. Zar, and C. N. von Ende. 1998. Field and Laboratory Methods for General Ecology, 4th ed. McGraw-Hill, Boston.

Darling, D. C., and L. Packer. 1988. Effectiveness of malaise traps in collecting Hymenoptera: the influence of trap design, mesh size, and location. Canadian Entomologist 120: 787-796.

Frank, J. H., J. P. Parkman, and F. D. Bennett. 1995. *Larra bicolor* (Hymenoptera: Sphecidae), a biological control agent of *Scapteriscus* mole crickets (Orthoptera: Gryllotalpidae), established in Northern Florida. Florida Entomologist 78: 619-623.

Gayubo, S. F., J. A. González, J. D. Asís, and J. Tormos. 2005. Conservation of European environments: the Spheciformes wasps as biodiversity indicators (Hymenoptera: Apoidea: Ampulicidae, Sphecidae and Crabronidae). Journal of Natural History 39: 2705-2714.

Gleason, H. A., and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada, 2nd ed. New York Botanical Garden, New York.

Hebard, M. 1917. The Blattidae of North America, north of the Mexican boundary. Memoirs of the American Entomological Society 2: 1-284.

Keefe, T. J., and E. P. Bergersen. 1977. A simple diversity index based on the theory of runs. Water Research 11: 689-691.

Krebs, C. J. 1999. Ecological Methodology, 2nd ed. Benjamin/Cummings, Menlo Park, CA.

Krombein, K. V. 1967. Trap-nesting wasps and bees: life histories, nests, and associates. Smithsonian Press, Washington, DC.

Krombein, K. V. 1979. Superfamily Sphecoidea, pp. 1573-1740. In Krombein, K. V., P. D. Hurd, Jr., D. R. Smith, and B. D. Burks, eds. Catalog of Hymenoptera in America North of Mexico, Vol. 2. Smithsonian Inst. Press, Washington, DC.

O'Brien, M. F. 1989. Distribution and biology of the sphecine wasps of Michigan (Hymenoptera: Sphecidae: Sphecinae). Great Lakes Entomologist 22: 199-217.

Pate, V. S. L. 1949. A minute on *Podium luctuosum*. Entomological News 60: 174.

Rau, P. 1928. The nesting habits of the wasp, *Chalybion caeruleum*. Annals of the Entomological Society of America 21: 25-34.

Simpson, E. H. 1949. Measurement of diversity. Nature 163: 688.

Steffan-Dewenter, I. 2002. Landscape context affects trap-nesting bees, wasps, and their natural enemies. Ecological Entomology 27: 631-637.

Townes, H. 1972. A lightweight malaise trap. Entomological News 83: 239-247.

Tscharntke, T., A. Gathmann, and I. Steffan-Dewenter. 1998. Bioindication using trap-nesting bees and wasps and their natural enemies: community structure and interactions. Journal of Applied Ecology 35: 708-719.

Willand, J. E. 2006. Diversity of ground beetles (Coleoptera: Carabidae) in relation to habitat characteristics and prescribed burning. M.S. Thesis, Western Illinois University, Macomb, Illinois, 34 pp.

Table 1. Numbers, species richness, rarefaction estimates of species richness (± SD), and species diversity of sphecine wasps captured in malaise traps in four habitats: prairie burned in spring 2005 (RBP – recently burned prairie), prairie last burned in spring 2004 (PBP – previously burned prairie), oak-hickory forest burned in spring 2005 (RBF – recently burned forest), and oak-hickory forest unburned for 5+ years (UF – unburned forest). Traps were operated continuously from 16 May to 24 October 2005 in Hancock Co., Illinois USA. Percentages do not sum to 100.0 due to rounding error.

Species	RBP	PBP	RBF	UF	Total	% of Total Wasps
Ammophila nigricans Dahlbom	1008	439	15	1	1463	73.9
Ammophila urnaria Dahlbom	42	11	1	0	54	2.7
Chalybion californicum (de Saussure)	20	7	1	0	28	1.4
Eremnophila aureonotata (Cameron)	154	42	12	3	211	10.7
Isodontia apicalis (F. Smith)	25	2	0	0	27	1.4
Isodontia mexicana (de Saussure)	22	8	1	0	31	1.6
Podium luctuosum F. Smith	3	0	0	4	7	0.4
Sphex ichneumoneus (L.)	6	0	0	0	6	0.3
Sphex nudus Fernald	122	17	0	0	139	7.0
Sphex pensylvanicus L.	15	0	0	0	15	0.8
Total Wasps Captured	1417	526	30	8	1981	100.2
Species Richness	10	7	5	3		
Rarefaction Estimate	10.0 ± 0.03	9.72 ± 0.49	4.89 ± 1.14	2.68 ± 0.93		
Simpson's Diversity	0.47	0.30	0.61	0.68		

Figure 1. Weekly collections of three species of sphecine wasps captured in malaise traps from May-October 2005 at Alice L. Kibbe Life Science Station, Hancock Co., Illinois USA.

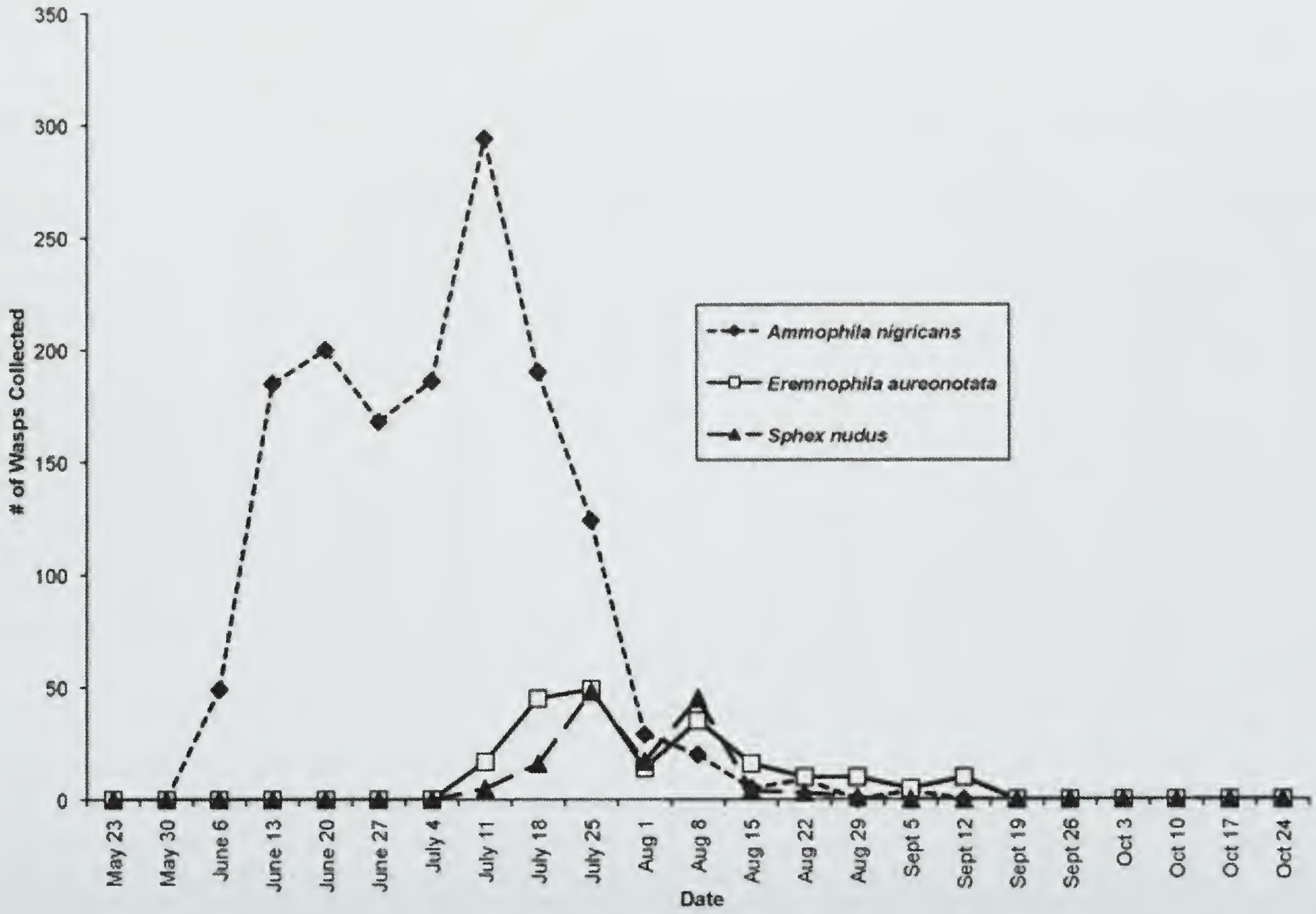
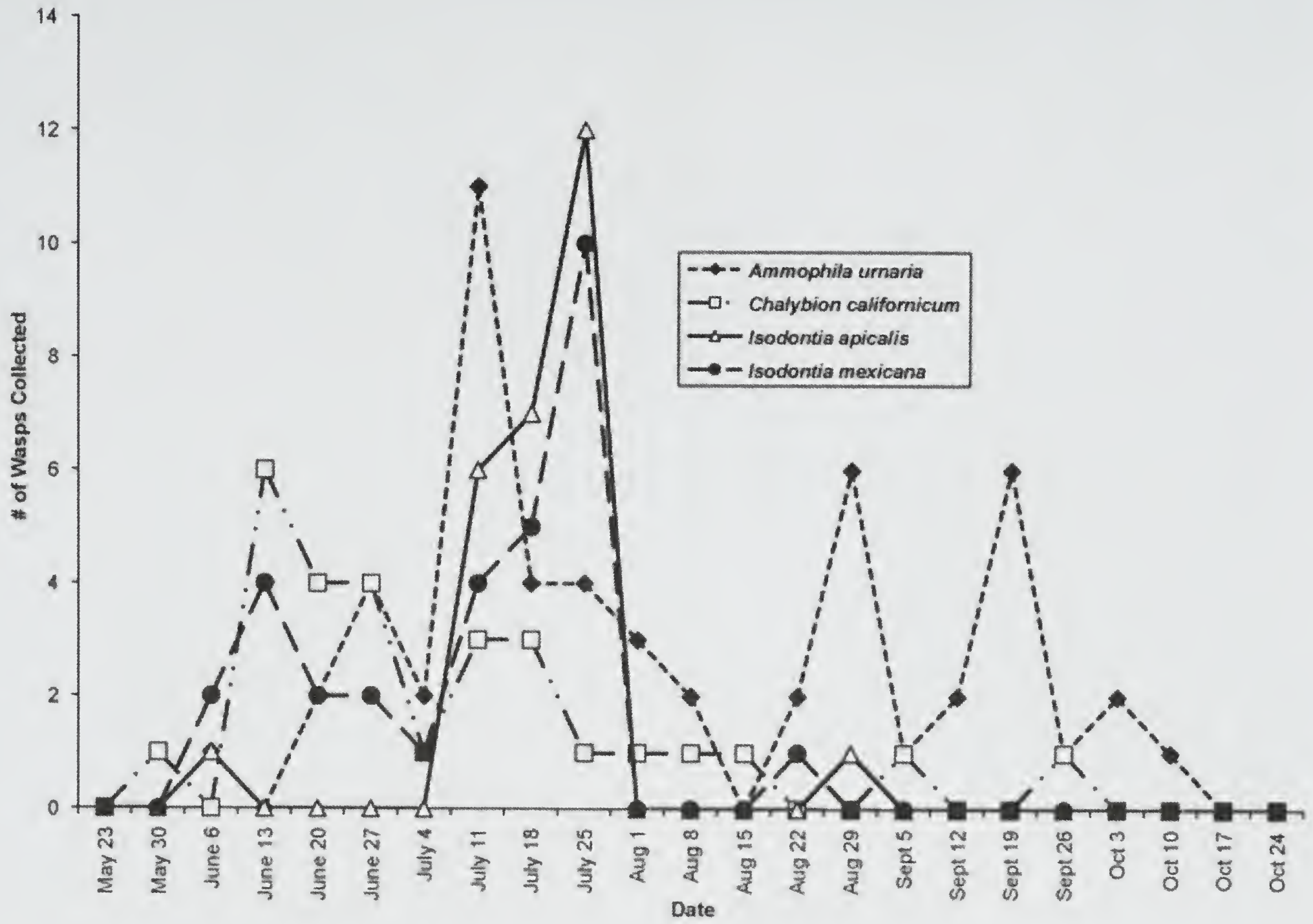


Figure 2. Weekly collections of four species of sphecine wasps captured in malaise traps from May-October 2005 at Alice L. Kibbe Life Science Station, Hancock Co., Illinois USA.



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Aspects of Raccoon Life History in West-Central Illinois

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ABSTRACT

Raccoons (*Procyon lotor*) were livetrapped and marked during 1989–1993 in an agriculturally dominated landscape in Brown County, west-central Illinois. Densities averaged 4.5 per km^2 with males dominating in the younger age classes (< 2 yrs) and females in older raccoons. Breeding rates averaged 46% for yearling females and 87% for adult females, with yearlings contributing about 20% of annual production. Juvenile survival rate averaged 74% between birth and 6 months. Both sexes continued to gain body weight into their 3rd year with males consistently heavier ($P < 0.03$) than females. Males lost body condition in winter while females remained in a more stable condition throughout the year. Dispersal behavior was more pronounced in males. Females of all age classes remained on the study area somewhat longer ($P > 0.05$) than males. Males were more likely to die from hunting and trapping causes while females were more likely than males to die of disease. Seasonal home ranges were similar for both sexes ($P < 0.05$) but male ranges averaged somewhat larger areas. We found no evidence that west-central Illinois raccoons were territorial as a paired removal experiment showed no attempt by the remaining member of each pair to make directed movements into a vacated range. Our study animals appeared to be above average in weight and condition, to be breeding at a level consistent with a healthy condition and to be generally under-harvested.

INTRODUCTION

Raccoons are adaptable and equally at home in urban and rural environments (Prange et al. 2004, Lotze and Anderson 1979). Raccoon populations are currently high in Illinois, the result of low exploitation by hunters and trappers and favorable habitat conditions (Gehrt et al. 2002, Bluett and Hubert 1992).

Aspects of raccoon life history have been previously investigated throughout Illinois. These reports have included the fate of translocated raccoons (Mosillo et al. 1999), a demographic comparison of rural and urban raccoons (Prange et al. 2003, 2004), and a general life history study (Stevens et al. 1995), all from northern Illinois. In addition, age specific reproductive rates were determined from harvested raccoons collected in west-central and northern Illinois (Fritzell et al. 1985) while reproductive cycles, aging techniques, and kit growth rates were examined from raccoons captured in west-central and east-central Illinois (Sanderson and Nalbandov 1973, Sanderson 1961, Montgomery 1964, 1968). Incidence of lead in raccoon livers collected in west-central and east-central Illinois were reported by Sanderson and Thomas (1961) and Stains (1961) examined winter temperature variations inside and outside raccoon tree dens in southern Illinois. Ellis (1964) reported one of the early uses of radio telemetry in delineated animal movements using raccoons captured in east-central Illinois. Demographic comparisons including sex ratios, age composition, litter size, and body weights among raccoons harvested in west-central, northern and southeastern Illinois were reported by Sanderson and Hubert (1981). Nielsen et al. (2007) surveyed the incidence of *Bayliscaris procyonis* and canine distemper virus in southern Illinois raccoons. Roy Nielsen and Nielsen (2007) examined relatedness and spacing among raccoons, and Wilson and Nielsen (2007) determined habitat selections by day bedded raccoons, both studies conducted in southern Illinois.

These studies determined the raccoon population in Illinois to be generally healthy and to have been increasing, particularly in urban areas. Because of their potential to affect disease transmissions among humans and domestic animals, as well as the often destructive nature of their feeding and denning behaviors, knowledge of raccoon life history traits remain important if we are to coexist with them in an increasingly urbanizing environment.

Previously published reports from our study of raccoon ecology in west-central Illinois have included a serological survey for infectious disease agents of raccoons (Mitchell et al. 1999a), an examination of aerobic microbial flora (Mitchell et al. 1999b), periodontal indices and the prevalence of cavities (Hungerford et al. 1999), raccoon survival (Mankin et al. 1999), and denning behavior (Nixon et al. 2001). Our objectives in this present report were to complete our examination of raccoon population dynamics and social relationships in a rural population exposed to hunting and trapping pressures in west-central Illinois.

STUDY AREA

The privately owned 23.4 km^2 study area was located in Brown County, Illinois and consisted of 59% row crops (corn [*Zea maize*] and soybean [*Glycine* max]), 13% fescue dominated pasture or forage (alfalfa [*Medicago sativa*]-clover [*Trifolium* sp.] or wheat [*Triticum aestivum*]), 25% shrub and second growth upland oak (*Quercus* sp.)-hickory (*Carya* sp.), 2% farmsteads, and a few farm ponds (0.01%). The study area was fixed by the boundaries of the Wells Creek watershed in Brown County. Pastures grazed by domestic livestock were scattered throughout the study area. Raccoons had access to waste grain, livestock feed and some garbage from scattered farmsteads. This area was

open for raccoon hunting and trapping each fall (November–January each year) and occurred on most farms on the study area.

METHODS

Livetrapping was undertaken each spring (March–June) and fall (August–October) on the study area from fall 1989 through fall 1993. Homemade box traps were baited with sardines and set for 10–30 days, with traps placed in areas likely to attract raccoons rather than placed in any type of regular configuration. Trap density varied with trapping success over the study area. Raccoon handling followed the animal protocol procedures outlined by the University of Illinois Institutional Animal Care and Use Committee. Captured raccoons were sedated using Telozol (Tiletamine HCL and Zolazepam HCL, Fort Dodge Labs, Inc, Fort Dodge, Iowa) given intramuscularly at 5 mg/kg of weight. Raccoons were allowed to remain in a trap until fully recovered from sedation and then released. Captured raccoons were ear-tagged using rotatable tags, sexed, weighed (first capture only as a juvenile, yearling, or adult), field aged (as birth year or after birth year), examined for external parasites, bled using heart puncture, measured for length and checked for tooth cavies and general oral condition. A fecal sample was also taken for parasite and disease diagnosis and a premolar tooth extracted from raccoons > 1 year old for aging using tooth cementum annuli (Grau et al. 1970). Females were checked for signs of nursing during the spring trapping period. Breeding rates of females on our study area were then compared with placental scar counts from west-central Illinois raccoons examined at the Peradi Brothers Fur and Wool Company, Farmington, IL by G. C. Sanderson during 1990–93 (G. C. Sanderson, Illinois Natural History Survey, personal communication 1995).

Without a rigorous trapping design, mark recapture analysis based on a JOLLY-SEBER analysis (Pollock et al. 1990) produced very high variances around a mean value. We used the minimum number of raccoons known present during each trapping period, developed from the age structure of captured raccoons each trapping period minus known dispersals and deaths.

We determined longevity of individuals on the study area based on recaptures and known mortality and dispersals reported to us by the public. When a marked raccoon disappeared from the area between trapping periods we used the midpoint between trapping efforts as an approximate disappearance date. Causes of mortality were also developed from necropsies of radio marked and eartagged raccoons.

Radio transmitters (Advanced Telemetry Systems, Isanti, MN) equipped with a motion sensitive switch were placed on 22 males (8 juveniles, 14 adults) and 24 females (7 juveniles, 4 yearlings, 13 adults). Radio marked raccoons were located using 2 truck-mounted, 8-element yagi antennas aligned in a null configuration. Each radio location was derived from 2–5 bearings taken from fixed locations generally less than 400 m apart scattered over both study areas. In summer, location error using >100 known radio-locations varied from a low of 77 m using 4 bearings to 147 m using 2 bearings. Location errors in winter fell between these bearings. Accuracy of locations was verified using transmitters placed at known locations throughout each study area, as well as a line-of-sight to a known transmitter location to align the antennas. These data indicated that rac-

coon locations could usually be accurately located within the 1-ha grids that covered the study area. Raccoons were radio located 2–4 times per week throughout the year usually from late afternoon to 2–4 hours post sunset, with locations taken < 60 min. apart. Locations produced by radio fixes were validated using the computer program LOCATE II (Nams 1990) and then assigned to a 1-ha grid. Radios emitting a mortality signal were located on foot using a hand antenna. Seasonal home ranges of radio marked raccoons were determined using the program RANGES IV (Kenwood 1990). Home range area was calculated using the harmonic mean method (Dixon and Chapman 1980) and was defined as the 75% outer convex polygon. Wandering-feeding movements of raccoons were determined by locating raccoons in a diurnal resting site and then relocating them periodically up to 4 hours after sunset. Known dispersal rates were calculated from movements of radio marked raccoons as well as from deaths of ear-tagged raccoons off the study areas.

Raccoons may or may not demonstrate sex related territorial behavior in Illinois (Gehrt and Fox 2004, Gehrt and Fritzell 1998). To examine this aspect of social behavior, we radio marked 10 adjacent adult raccoons (3 male pairs, 2 female pairs), 4 pairs on the study area and 1 pair on an adjacent state park and collected at least 6 months of movements (including simultaneous locations if possible). We then removed one member of each pair and monitored the movements of the remaining raccoon to see if home range locations changed after removal of the neighbor. Changes in home range shapes of remaining adults after removal of the neighbor were measured to see what portion remained constant, were abandoned, or were totally new in relation to the neighbor's home range.

Radio locations for all radio marked raccoons were grouped into periods bounded by important behavioral or physiological changes that potentially affect movement patterns. The winter period (1 December–15 March) was one of weight loss, communal denning, breeding, and reduced movements during inclement weather. Spring (16 March–31 May) brought weight gains for both sexes and birth and early kit growth. Summer (1 June–30 September) was a time of rapid kit growth, increasing movements of females and young, and abundant food sources. Fall (1 October-30 November) included weight gains prior to winter and partial or complete family breakup.

Comparisons between the sexes and ages of raccoons were examined using Oneway Anova and Chi-squared analysis where appropriate (Sokal and Rohlf 1995). Means are reported with standard errors.

RESULTS

Raccoon numbers

Between fall 1989 and fall 1993, we captured 440 individual raccoons on the study area (249 males and 191 females). Usable data were obtained for this report only from 346 raccoons (208 males and 138 females), with the remainder dying early, dispersing, or never located again. The minimum known number of raccoons present ranged from an average of 115 in the fall (range 71–171) to 90 in the spring (range 58–124) (4 seasons), indicating a density of about 4.5 raccoons km^2 on the study area during our trapping program.

Pooling captures from all 4 years, sex ratios were significantly different between age classes in the spring (X^2 = 14.2, df = 4,225, P < 0.006) due to a preponderance of males in the yearling and 2-year-old classes and more abundant females in the older classes (Table 1). This same pattern repeated in the fall trapping but the sex ratios were not significantly different (P = 0.08). Higher mortality and dispersal behaviors for males likely accounted for the differences in sex ratios in the older age classes (Table 1).

Breeding rates and recruitment

Yearling breeding rates, evidenced by nursing, averaged 46% on the study area (range 33–54%, N = 41) and were lower than those recorded using placental scar counts at the Peradi Brothers Fur and Wool Company for raccoons killed in west-central Illinois for 1990–1993 (50%, N = 32, range = 33–67%) (G. C. Sanderson, Illinois Natural History Survey, personal communication 1995). Adult female breeding was also lower (87%, N = 60, range = 80–93%) than for females examined at the fur house (98% parous, N = 53, range = 75–100%) and other areas in Illinois (Sanderson and Hubert 1981). A higher breeding rate based only on placental scars would be expected because of in utero mortalities and, in the raccoon, the presence of scars from previous pregnancies (Sanderson and Nalbandov 1973).

A crude estimate of kit recruitment to weaning age was possible using placental scars found in females examined at the fur house compared with the number of juveniles per breeding female captured in the fall on our study areas. The losses of young (death and dispersals) averaged 26% for the 4–6 months interval between birth and capture. A trap bias favoring juvenile captures is at least partially offset by the dispersal tendencies for post-weaned young.

Body weights and condition

Body weights were not obtained from all captures because of time constraints (multiple captures/day), physical deformities, or an obvious appearance of poor condition. Older age classes weighed more into their 3rd year (Table 2). Age class body weights (juveniles, yearlings, and adults) did not differ between seasons for either sex. Spring and fall weights of male yearlings were significantly heavier than female yearling weights (P < 0.03), and two-year old males were heavier than two-year old females in the fall (P < 0.007) but not in the spring. Three-year old and older male raccoons were significantly heavier than females during both trapping seasons (P < 0.01). As reported by Mankin et al. (1999), there were no significant differences in condition indices (body mass/body length^3 X 100,000) between males and females within any age group (P > 0.17) in this population. Male condition significantly increased from spring to fall (F = 4.8, P < 0.001), while adult female condition remained fairly constant throughout the year (P = 0.17) (Mankin et al. 1999).

Known dispersals

Dispersal behavior declined slowly in males as they matured but declined abruptly in females after their first year of life. Yearling and older males were much more likely to disperse away from their natal area than females (Table 4). Dispersal tendencies of both sexes of juveniles were similar. Males were also more likely to disperse farther away than were females. Three males were killed > 32 km from the study area, a juvenile shot 40

km east, a yearling shot 35 km northwest, and a yearling trapped 42 km northwest of the study area.

Longevity and mortality

Females remained on the study area longer than males for all 3 age classes, but the differences were not significant ($P > 0.05$). Female juveniles averaged 8.5 ± 1.0 months (N = 51) on the study area while male juveniles averaged 6.9 ± 0.9 months (N = 68). For yearlings, females averaged 8.9 ± 1.2 months (N = 51) and males 6.3 ± 0.8 months (N = 78). Adult females averaged nearly 1 year on the study area (11.1 ± 1.5 months; N = 50), and adult males averaged 8.2 ± 1.3 months.

A total of 10 males and 14 females survived to 5 years and older. Three males reached 6 years and 2 reached 7 years. For females, 9 reached 6 years and 1 reached 7 years when killed. Female #838, tagged as a juvenile in fall 1993, was killed at the age of 12 years on the highway in December 2005 close to its capture site.

Hunting and trapping, highway accidents, and various diseases were the leading causes of known mortality for raccoons on the study area (Table 4). Hunting and trapping losses accounted for 69% of the known mortality. Proportionate losses to various mortality agents were about the same for both sexes, with males more likely to be harvested and females to die of disease.

Home ranges and local movements

For each sex, there was no significant difference in home range size among seasons ($P > 0.05$). Females averaged smaller home ranges compared to males throughout the year, except in fall for > 2 year old females (Table 5). Seasonal home range averages were similar between sexes ($P > 0.05$).

Wandering-feeding movements of radio marked raccoons averaged less for males (212 m/hr) than for females (277 m/hr) during winter ($P < 0.05$) (Table 6). Movements of both sexes were similar during the remainder of the year. During the breeding season there were 13 days where males were located on consecutive days. For 10 of these days movement was < 100 m. Three other daily movements were 860 m, 861 m, and 608 m, respectively. For females during the same period, there were 12 days with no movement between locations and 1 movement of 3,111 m between day beds. During the parturition period, 2 movements of females were < 100 m, and one movement totaled 223 m. During summer, 8 movements were < 100 m, and 9 movements averaged 582 m with a range of 141–1,334 m.

Social relationships

For the 5 pairs of adjacent raccoons > 1 year old (2 pairs of females, 3 pairs of males) that were monitored pre-and post removal of one member, all but the parous pair of females (#299 and #499) showed significant home range overlap (> 50%) during the pre-removal phase of study (total telemetry locations all pairs = 2,145).

For the 3 adult males remaining post-removal, 2 males increased their home range, and 1 decreased the size of its range. All 3 males moved into new areas and changed their centers of activity after removal of their neighbor, but they made no directed moves into the

newly vacated neighbor's range. The change in male activity centers averaged 500 m (range 200–940 m) after neighbor removal with an average of 24% of each home range into a new area (range = 9.5–33%). Only male #670 moved into the previous neighbor's range to any extent (22% of the post removal range). Prior to removal of a neighbor, males did not socialize with each other to any extent in winter or spring (mean distance apart averaged between 232–276 m when simultaneously radio-tracked, locations = 211). During summer and fall, these males were occasionally located within the same hectare (1 pair were together about 30% of the time [N = 16 locations], another pair 20% of the time [N = 23 locations]) but these pairs averaged between 206–403 m apart.

Both remaining females increased their range after removal of their neighbor by occupying totally new territory (not part of the previous neighbor's range) averaging 31% of the post-removal home range. We also found that our 2 female pairs did not socialize during the year, averaging between 370–833 m apart (N = 4–16 simultaneous locations per season). These changes in home range observed over time were mimicked for our other radio marked samples of both sexes, with seasonal changes common as raccoon aged and neighbors were killed or dispersed. It should be pointed out that we did not control for new adults moving into the vacated ranges, for any subadults maturing in the vacated ranges, and for any unmarked and unknown adults that may have been present in both pre-and post-removal home ranges.

DISCUSSION

Rural raccoons in west-central Illinois appeared to be above average in weight with males heavier than females, were breeding at a level consistent with a healthy population (Fritzell et al. 1985), and were relatively stable in numbers during our study. Body weights continued to increase at least into the third year, a reflection of good nutrition and success at locating food sources. Annual survival rate of radio marked raccoons averaged 0.74 ± 0.07 in this population (Mankin et al. 1999). Raccoon density on our study area was similar to rural populations studied in McHenry County (Prange et al. 2004) but lower than densities for 2 areas in Lee County (Stevens et al. 1995), both in northern Illinois. Rural populations appear to be consistently smaller than suburban or urban populations, the result of lower survival and site fidelity for both sexes in rural habitats (Prange et al. 2004). Females survived on the area longer than males, were less likely to be killed by hunters and trappers, and remained in good condition throughout the year. Higher mortality and dispersal behavior for males likely accounted for the difference in sex ratios in the older age classes. Males occupied larger home ranges, were more likely to be killed, and lost condition during the winter months. Family breakup occurred both in the fall and spring, but families often remained together throughout the winter (Gehrt and Fritzell 1998). For carnivores, male home range size appears to be influenced by the spatial distribution of food supplies and female numbers and distribution, while range size of females conforms more to resource distribution (Sandell 1989).

The overlap of home ranges (> 50%) for our paired raccoons prior to removal of one of each pair indicated a lack of exclusive territoriality. It could be argued that the lack of movement into the vacated range by the surviving raccoon was the result of aggression by the resident prior to removal. However, based on simultaneous radiotracking prior to any removal, raccoons were seldom together and gave no indication that aggression had

occurred (rapid movements away from each other, no injuries were observed). The male pairs did travel together occasionally in summer and fall.

There seems to be a mutual avoidance behavior most of the year for both sexes with males more likely to associate at least occasionally. Radio marked raccoons were almost always observed feeding alone except for mother and young associations. Our marked raccoons did seek out communal feeding sites throughout the year and denning sites in winter, and a dominance hierarchy was evident at these sites (visual observations).

Gehrt and Fox (2004) reported that raccoons in northern areas of North America were considered solitary in habit with spatial overlap greatest during the winter months. However, Pitt et al. (2008) found male raccoons organized as 2-member coalitions that were territorial in southern Manitoba, Canada, a population at the edge of their continental distribution. Each of these coalitions overlapped several female ranges. At least some males in the southern United States, also manifest territorial behavior to other males. Gehrt and Fritzell (1997) found some males were arranged in intra-sexual groups in all seasons in south Texas. Chamberlain and Leopold (2002) also noted some male groups manifested territorial behavior in Mississippi. Male home ranges are usually larger than female ranges and overlap several female ranges (Barding 2006, Prange et al. 2004, Walker and Sunquist 1997). Females throughout their range occupy overlapping home ranges but, except at communal feeding sites, do not socialize with other females (Fritzell 1978, Barding 2006, Gehrt and Fox 2004, Suzuki et al. 2003, Pitt et al. 2008). We captured adults of both sexes at the same livetrap location during each trapping period, indicating the raccoons were occupying overlapping home ranges on our study area. Roy Nielsen and Nielsen (2007) reported that many raccoon litters had multiple sires indicating > 1 male had access to these estrous females. While we were unable to demonstrate any obvious terroritial behavior among west-central Illinois raccoons, such behavior remains a possibility.

Our limited radio tracking bed-to-bed of both sexes obviously underestimated daily movements. Summer bed-to-bed movements averaged 3.2 km for adult males, 2.4 km for yearling males, 1.6 km for adult females, and 1.5 km for barren females in North Dakota (Greenwood 1982). Mech et al. (1966) and Barding (2006) reported movements closer to our findings, ranging from 232–956 m.

Without a resurgence in raccoon fur prices, hunting and trapping will likely not materially reduce raccoon numbers in Illinois, but any reduction in numbers would aid in reducing raccoon densities. Clark et al. (1989) determined that harvest levels must exceed 40% of the raccoon population to become additive to overall mortality and to significantly affect subsequent densities. There is no indication that harvests in Illinois are approaching this level at present (Bluett and Hubert 1992). Local populations are also subject to canine distemper viral outbreaks, generally on a 3–5 year cycle (Mitchell et al. 1999a, Lotze and Anderson 1979). These localized reductions are usually quickly replenished by dispersers from unaffected populations, usually juveniles and adult males (Barton and Roth 2007). The steady increase in the spread of suburban housing throughout Illinois is also increasing human and raccoon interactions (Prange et al. 2004) and causing a resultant upsurge in nuisance complaints (Bluett 1995).

ACKNOWLEDGEMENTS

We are grateful to J. Seets, M. A. Mitchell, and G. R. Lang for their assistance in this study. We also thank the landowners of the Wells Creek watershed for allowing us access to their farms. G. F. Hubert, Jr., Illinois Department of Natural Resources, provided financial and logistical support. This study was supported by the Federal Aid in Wildlife Restoration Project W-104-R, the Illinois Department of Natural Resources, the U.S. Fish and Wildlife Service, and the Illinois Natural History Survey cooperating.

LITERATURE CITED

Barding, E. E. 2006. Movements and habitat selection of raccoons in a rural Illinois landscape. M.S. Thesis, Eastern Illinois University, Charleston. 41pp.

Barton, B. T. and J. D. Roth. 2007. Raccoon removal on sea turtle nesting beaches. J. Wildl. Manage. 71:1234–1237.

Bluett, R. D. 1995. Nuisance wildlife control in Illinois—1994 summary. Illinois Department of Conservation. Furbearer Program Management Note 95-1:1-4.

Bluett, R. D., and G. F. Hubert, Jr. 1992. Fur harvest survey, 1991–92. Ill. Dept. Conserv., Fed. Aid in Wildl. Restor., Proj. W-99-R. 10pp.

Chamberlain, M. J. and B. D. Leopold. 2002. Spatio-temporal relationships among adult raccoons (*Procyon lotor*) in central Mississippi. Amer. Midl. Nat. 148:297–308.

Clark, W. R., J. J. Hasbrouck, J. M. Kienzler, and T. F. Glueck. 1989. Vital statistics and harvest of an Iowa raccoon population. J. Wildl. Manage. 53:982–990.

Dixon, K. R. and J. A. Chapman. 1980. Harmonic mean measure of animal activity areas. Ecology 61:1040–1044.

Ellis, R. J. 1964. Tracking raccoons by radio. J. Wildl. Manage. 28:363–368.

Fritzell, E. K. 1978. Aspects of raccoon (*Procyon lotor*) social organization. Can. J. Zool. 56:260–271.

Fritzell, E. K., G. F. Hubert, Jr., B. E. Meyen, and G. C. Sanderson. 1985. Age specific reproduction in Illinois and Missouri raccoons. J. Wildl. Manage. 49:901–905.

Gehrt, S. D. and E. K. Fritzell. 1997. Sexual differences in home ranges of raccoons. J. Mammal. 78:921–931.

Gehrt, S. D. and E. K. Fritzell. 1998. Resource distribution, female home range dispersion, and male spatial interactions: group structure in a solitary carnivore. Anim. Behav. 55:1211–1227.

Gehrt, S. D., G. F. Hubert, Jr., and J. A. Ellis. 2002. Long term population trends of raccoons in Illinois. Wildl. Soc. Bull. 30:457–463.

Gehrt, S. D. and L. B. Fox. 2004. Spatial patterns and dynamic interactions among raccoons in eastern Kansas. The Southwest. Nat. 49:116–121.

Grau, G. A., G. C. Sanderson, and J. P. Rogers. 1970. Age determination of raccoons. J. Wildl. Manage. 34:364–372.

Greenwood, R. J. 1982. Nocturnal activity and foraging of prairie raccoons (*Procyon lotor*) in North Dakota. Am. Midl. Nat. 107:238–243.

Hungerford, L. L., M. A. Mitchell, C. M. Nixon, T. E. Esker, J. B. Sullivan, R. Koerkenmeier, and S. M. Marretta. 1999. Periodontal and dental lesions in raccoons from a farming and a recreational area in Illinois. J. Wildl. Diseases 35:728–734.

Kenwood, R. 1990. Rangers IV: software for analyzing animal location data. Institute of Terrestrial Ecology, Wareham, United Kingdom. 33pp.

Lotze, J. H. and S. Anderson. 1979. *Procyon lotor*. Mammal. Species 119. Amer. Soc. Mammal. 8pp.

Mankin, P. C., C. M. Nixon, J. B. Sullivan, T. L. Esker, R. Koerkenmeier, and L. L. Hungerford. 1999. Raccoon (*Procyon lotor*) survival in west-central Illinois. Trans Ill. Acad. Sci. 92:247–256.

Mech, L. D., J. R. Tester, and D. W. Warner. 1966. Fall daytime resting habits of raccoons as determined by telemetry. J. Mammal. 47:450–466.

Mitchell, M. A., L. L. Hungerford, C. Nixon, T. Esker, J. Sullivan, R. Koerkenmeier, and J. P. Dubey. 1999a. Serological survey for selected infectious disease agents in raccoons from Illinois. J. Wildl. Diseases 35:347–355.

Mitchell, M. A., L. L. Hungerford, C. Nixon, T. Esker, J. Sullivan, R. Koerkenmeier, and J. P. Dubey.. 1999b Survey of the oral aerobic microbial flora of the raccoon. The NWRA Quarterly Journal. 3pp.

Montgomery, G. G. 1964. Tooth eruption in preweaned raccoons. J. Wildl. Manage. 28:582–584.

Montgomery, G. G. 1968. Pelage development of young raccoons. J. Mammal. 49:142–145.

Mosillo, M., E. J. Heske, and J. D. Thompson. 1999. Survival and movements of translocated raccoons in northcentral Illinois. J. Wildl. Manage. 63:278–286.

Nams, V. O. 1990. Locate II: user's guide. Pacer, Truro, Nova Scotia, Canada. 84pp.

Nielsen, C. K., A. Defore, and E. Bade. 2007. Survey of *Bayliscaris procyonis* and canine distemper virus in southern Illinois. Trans. Illinois Acad. Science 100:169–176.

Nixon, C. M., J. B. Sullivan, T. L. Esker, R. Koerkenmeier, and G. Hubert, Jr. 2001. Den use by raccoons in west-central Illinois. Trans. Ill. Acad. Sci. 94:59–65.

Pitt, J. A., S. Lariviere, and F. Messier. 2008. Social organization and group formation of raccoons at the edge of their distribution. J. Mammal. 89:646–653.

Pollock, K. H., J. D. Nichols, C. Browne, and J. E. Hines. 1990. Statistical inference for capture-recapture experiments. Wildl. Monogr. 107. The Wildlife Society, Washington, D.C. 97pp.

Prange, S., S. D. Gehrt, and E. P. Wiggers. 2003. Demographic factors contributing to high raccoon densities in urban landscapes. J. Wildl. Manage. 67:324–333.

Prange, S., S. D. Gehrt, and E. P. Wiggers. 2004. Influences of anthropogenic resources on raccoon (*Procyon lotor*) movements and spatial distribution. J. Mammal. 85:483–490.

Roy Nielsen, C. L. and C. K. Nielsen. 2007. Multiple paternity and relatedness in raccoons *Procyon lotor*. J. Mammal. 88:441–447.

Sandell, M. 1989. The mating tactics and spacing patterns of solitary carnivores. Pp. 164–182. In J. L. Gittleman (ed). Carnivore behavioral ecology and evolution. Cornell University Press, Ithica, N.Y.

Sanderson, G. C. 1961. Techniques for determining age of raccoons. Illinois Nat. Hist. Survey Biol. Note 45:1–16.

Sanderson, G. C. and R. M. Thomas. 1961. Incidence of lead in livers of Illinois raccoons. J. Wildl. Manage. 25:160–168.

Sanderson, G. C. and A. V. Nalbandov. 1973. The reproductive cycle of the raccoon. Ill. Nat. Hist. Surv. Bull. 31:29–85.

Sanderson, G. C. and G. F. Hubert, Jr. 1981. Selected demographic characteristics of Illinois (U.S.A.) raccoons (Procyon lotor). Pp. 487-513. In J. A. Chapman and D. Pursley eds. Proc. World-wide Furbearer Conf., Frostburg, Md.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry. W. H. Freeman and Co., San Francisco, CA.

Stains, H. J. 1961. Comparison of temperatures inside and outside two tree dens used by raccoons. Ecology 42:410–413.

Stevens, J., C. Nixon, and J. Suver. 1995. Some aspects of raccoon life history in Lee County, Illinois. Trans. Illinois Acad. Sci. 88:49–59.

Suzuki, T., T. Aoi, and K. Maekawa. 2003. Spacing pattern of introduced female raccoons (*Procyon lotor*) in Hokkaido, Japan. Mammal Study 28:121–128.

Walker, S. and M. Sunquist. 1997. Movement and spatial organization of raccoons in north-central Florida. Florida Field Nat. 25:11–21.

Wilson, S. E. and C. K. Nielsen. 2007. Habitat characteristics of raccoon daytime nesting sites in southern Illinois. Amer. Midl. Nat. 157:175–186.

Table 1. Sex and age of raccoons livetrapped in Brown County, Illinois, 1989–93. Numbers are summaries of all raccoons captured each year.

	Fall				Spring			
Age (in years)	Males	%	Females	%	Males	%	Females	%
< 1	76	42.4	66	48.9	—[a]	—	—	—
1	47	26.2	22	16.3	58	47.1	36	32.7
2	34	18.9	18	13.3	38	30.9	27	24.5
3	9	5.0	11	8.1	16	13.0	19	17.3
4	6	3.3	8	5.9	5	4.1	12	10.9
≥ 5	7	3.9	10	7.4	6	4.9	16	14.5
Total	179		135		123		110	

[a]juveniles too young to livetrap

Table 2. Body weights of raccoons at first capture in Brown County, Illinois 1989–93.

		Males			Females		
			Mean Weight			Mean Weight	
Age (in years)	Season	N	Kg	S.E.	N	Kg	S.E.
< 1	Fall	47	3.7	0.14	35	3.5	0.15
1	Spring	33	5.3	0.14	28	4.5	0.12
	Fall	18	5.5	0.19	8	4.7	0.21
2	Spring	19	5.7	0.26	7	5.1	0.30
	Fall	16	6.5	0.29	8	5.1	0.28
≥3	Spring	3	6.7	0.21	12	5.4	0.22
	Fall	3	7.6	0.23	7	5.4	0.30

Table 3. Dispersal behavior of raccoons livetrapped in Brown County, Illinois 1989–93.

			Known Dispersal	
Age (in years)	Sex	Number	N	Percent
< 1	Male	16	5	31.2
	Female	12	3	25.0
1	Male	30	10	33.3
	Female	24	1	4.2
≥2	Male	71	10	14.1
	Female	57	3	5.3

Table 4. Known causes of mortality for a marked sample of raccoons in Brown County, Illinois 1989–93.

	Females					Males				
	Juvenile	Yearling	Adult	Total	%	Juvenile	Yearling	Adult	Total	%
Harvest	11	9	17	37	67.3	11	15	27	53	70.7
Highway	3	2	3	8	14.5	0	3	7	10	13.3
Disease	0	1	8	9	16.4	0	1	6	7	9.3
Dog	0	0	1	1	1.8	0	0	3	3	4.0
Misc[a]	0	0	0	0	0	0	0	2	2	2.7
Total	14	12	29	55		11	19	45	75	

[a]one electrocuted, one poisoned

Table 5. Seasonal home ranges of radio-marked raccoons in Brown County, Illinois, 1989–93. Home ranges are defined as the 75% outer convex polygon (Dixon and Chapman 1980).

				Home Range (ha)		
Age (in years)	Season	Sex	N	Mean	S.E.	Range
< 1	Winter[a]	Male	6	59.9	15.2	16–115
		Female	8	26.6	4.9	7–47
1	Spring	Male	5	45.8	17.5	12–108
		Female	5	13.1	2.3	7–20
	Summer	Male	3	60.2	14.7	31–78
		Female	9	23.7	3.1	9–37
	Fall	Male	2	37.0	13.0	24–50
		Female	6	21.7	4.5	12–39
≥ 2	Winter	Male	11	32.0	7.4	7–82
		Female	4	22.1	5.7	12–35
	Spring	Male	7	33.8	6.8	11–71
		Female	7	24.0	9.2	3–71
	Summer	Male	18	54.3	6.0	16–109
		Female	14	51.6	12.1	15–186
	Fall	Male	18	40.1	4.2	11–88
		Female	10	44.1	7.4	17–90

[a] Winter = 1 Dec–15 March; Spring = 16 March–31 May; Summer = 1 June–30 September; Fall = 1 October–30 November.

Table 6. Raccoon movements (m/hour) between the location of a diurnal resting site and the last radio location taken 3–4 hours after sunset.

Season	Sex	Number of raccoons	Number of locations	Meters per hour	
				Mean	S.E.
Winter[a]	Male	4	30	212.3	3.5
	Female	3	34	276.6	4.3
Spring	Male	6	47	296.8	4.1
	Female	7	30	260.2	5.2
Summer	Male		----- No Data -----		
	Female	11	117	193.4	1.3
Fall	Male	4	10	372.8	14.2
	Female	3	6	373.3	15.7

[a] Winter = 1 Dec–15 March; Spring = 16 March–31 May; Summer = 1 June–30 September; Fall = 1 October–30 November.

BOOK REVIEW 2009 - #1

Hay, Robert K.M. and John R. Porter. The Physiology of Crop Yield. 2006. Second Edition. viii + 314 pages; tables; figures; one photomicrograph; one scanning electron micrograph; references; index. Blackwell Publishing Professional, Ames, Iowa. ISBN-13: 978-14051--0859-1. Soft Cover. Price: U.S. $59.99. Available from: Blackwell Publishing Professional, PO Box 570, Ames, IA 50010-0570

Since I did not have the benefit of using the earlier edition of The Physiology of Crop Yield, the present edition is the first edition I examined. My first quick read through its pages gave me the impression that it is a valuable book.

After the excellent introductory remarks in the first chapter, chapter two introduces the reader to the fascinating relationships between form and function of plant structures. The treatment of indeterminate growth (formation of more leaves and growth of stem after flowering) of Soybean (*Glycine max*) is succinctly done, explaining the difference between the indeterminate stem growth and indeterminate leaf appearance. Similar specific detailed analyses are provided for Potato (*Solanum tuberosum)* and Corn (= Maize; *Zea mays)* that will be of particular interest to students and professionals in the plant sciences in the United States, especially the Midwest.

The next two chapters (three and four) deal with solar radiation interception and photosynthesis. The discussion has a special emphasis on leaf area index and canopy architectural expansion in relation to solar radiation. Using a systems biology approach, the authors examine photosynthesis at three structural levels -- the cell, leaf, and canopy. Distinctive photosynthetic responses in terms of radiation use efficiency and the accumulation of sugars at these three levels are discussed. The authors are aware that there has been criticism concerning the utilization of radiation use efficiency for this purpose by some researchers. On the other hand, they made efforts to explain the differences in net photosynthesis at the cell, leaf, and canopy levels with respect to leaf growth and expansion, branching, and canopy architecture and expansion.

The next chapter (five) deals with the release of carbon dioxide during respiration. The relations among respiration, photosynthesis, synthesized biomass, and environmental factors are highlighted. Utilization of different substrates for respiration in relation to maintenance and growth of plants are treated in detail. Calculations of respiration associated with growth, enzyme functions (such as nitrate reductase), nitrate uptake, ion uptake, and phloem loading are explained. In addition, the authors briefly discuss the effects of elevated carbon dioxide on respiration and dry matter production. In C_3 plants, dry matter production increases with some increases in carbon dioxide levels, thereby providing more substrates for respiration.

Chapter six deals with the dry matter distributions among different plant structures not only their harvested parts. In essence, this is a detailed discussion of source-sink relationships. The relationship between ontogeny and pattern of partitioning of dry matter in relation to the expanding leaves is explored. Moreover, the role of source and sink limitations on yields is considered with specific emphasis on selected cereals – Corn (*Zea mays*), Barley (*Hordeum vulgare*), and Wheat (*Triticum aestivum*); as well as the tuber

crop, Potato (*Solanum tuberosum*). Additionally, the discussion on assimilate partitioning in grassland plants is very useful with the present trends towards developing bio-fuel crops.

Chapter seven deals with the consequences of limiting factors on crop yield. Two major limitations are considered – water and nitrogen. Water use efficiency and crop yield under limited water supply is discussed. A considerable discussion on drought avoidance by plants is provided, but drought tolerance is discussed only briefly. The acquisition of water and water use efficiency and the acquisition of nitrogen and nitrogen use efficiency in crop yield are dealt with in detail. Perhaps I am particularly interested, but I was slightly disappointed not to see any treatment of other soil factors such as salinity problems. Water deficiency and salt stress are interrelated and thus, a discussion of salt stress might well have been considered.

The next two chapters (eight and nine) deal with the physiology of crop quality and problems in modeling of crop growth and yield. Three models – AFRC2 for Wheat, CROPGRO for Soybean and the Muchow and Sinclair Model for Maize are discussed in detail. Also, the authors give the readers who are not familiar with modeling a thorough explanation of how these models are derived and what their limitations are. I was happy to see that the authors, themselves, expressed a cautionary note on the limitations of models in chapters three and four.

The last chapter, albeit a small one, is noteworthy in providing suggestions for future research. The potential for increasing crop yield with lower inputs and more research on cropping systems under various climatic conditions are mentioned. Also, a closer working cooperation among molecular biologists and crop physiologists is suggested, which most of us would strongly support.

Except for a minor disappointment in not seeing the effects of salt stress on crop yield covered, I certainly enjoyed the book. The authors have succeeded in presenting the subject matter in a thorough and concise manner. According to the authors, this book is meant for senior undergraduate students, but I recommend it to graduate students of crop physiology as well. In addition, agriculturists, agronomists, and regional land use planners need to have The Physiology of Crop Yield readily available.

Reviewer: Devi Prasad V. Potluri, Department of Biological Sciences, Chicago State University, Chicago, Illinois 60628

The 101st Annual Meeting
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Keynote Address

Dr. Richard E. Sparks
Director of Research
National Great Rivers Research and Education Center
Godfrey, Illinois

What makes a river "great"? Certainly the rivers where civilization began, such as the Nile, the Indus, the Tigris, and the Euphrates, qualify as "great" in human terms and warrant some explication of the link between these rivers and the rise of agriculture, writing, complex societies, and monumental architecture. These rivers were characterized by seasonal floods which inundated floodplains, thereby renewing the fertility of the soil and enabling people to practice agriculture and stay in one place for hundreds to thousands of years. Elsewhere, people had to move on when the fertility of the soil was exhausted. Although early civilizations capitalized on predictable seasonal floods by harvesting natural resources and later practicing a flood-adapted form of agriculture, they were also subject to occasional great floods and droughts that disrupted food production and destroyed lives and property.

Now the situation at first appears to be quite different. Virtually all the large rivers in the tropic and temperate parts of the world are regulated by dams and many of the floodplains and deltas are leveed and developed, not just for dryland agriculture, but also for industrial and municipal uses. Despite these technological achievements, severe problems have developed, including: recent damaging floods around the world (including two events in 2008 in the Upper Mississippi River Basin); expansion of hypoxic zones at river mouths; subsidence and erosion of river deltas; and decline or loss of many species, including commercially valuable fishes and charismatic species, such as the Yangtze River dolphin.

The new National Great Rivers Research and Education Center, founded by the University of Illinois, the Illinois Natural History Survey, and Lewis & Clark Community College, is partnering with other colleges and universities, government agencies, businesses, and non-governmental organizations to develop new, collaborative approaches to sustain ecological services of great rivers and help people live safely and well with these fascinating and dynamic ecosystems.

NOTES

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Old Field Succession and *Schizachyrium scoparium* at Sand Prairie-Scrub Oak Nature Preserve, Mason County, Illinois

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ABSTRACT

Schizachyrium scoparium, a dominant species of mature dry sand prairies in Illinois, is not found in the early stages of old field succession. A 30-year-old abandoned field at Sand Prairie-Scrub Oak Nature Preserve, Mason County, Illinois, when surveyed in 2000, was dominated by *Eragrostis trichodes* [importance value (I.V.) of 97.4], with *Strophostyles helvula* (I.V. 43.7) and *Monarda punctata* (I.V. 16.9) ranking second and third in I.V. (possible 200), while *Schizachyrium scoparium* was absent. When the authors revisited this field in the summer of 2008 *Schizachyrium scoparium* had become the dominant grass (I.V. 66.8), *Eragrostis trichodes* had dropped to fifth in importance (I.V. 11.1), while *Strophostyles helvoula* maintained second (I.V. 33.3). *Schizachyrium scoparium* entered, and established dominance in the old field sometime after the 2000 survey. The reasons for these changes are not known, but may be related to the soil mycorrhizal community.

INTRODUCTION

In pre-settlement times dry sand prairies were common on the upper slopes and ridges of dunes and other dry areas throughout the Illinois River sand deposits. Soils of dry sand prairie communities lacked a dark A horizon, and the grasses, most of which were bunch-grasses, rarely exceeded 1 m in height. In the absence of recurring fires dry sand prairies developed into dry sand savannas (White and Madany 1978). Gleason (1910) was the first to quantify the species composition of the Mixed Consocies of the Bunch-Grass Association, a classification that corresponds to the dry sand prairie community of White and Madany (1978). Gleason (1910) described this association as being dominated by native bunch-grasses and sedges with most other species restricted to the areas of bare sand between bunch-grasses.

A few dry sand prairie communities have been studied in the Illinois River sand deposits. These studies involved mature dry sand prairie communities that had been subjected to some present day minor disturbances and probably past grazing, but were never culti-

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vated. In these dry sand prairies *Schizachyrium scoparium* (little bluestem) was the dominant bunch-grass species, commonly accounting for a third to half of the importance value (Phillippe et al. 2004, McClain et al. 2005, 2008). One study, however, also involved successional communities of known age that were once cultivated (McClain et al. 2008). That study involved a 30-year-old field that was cultivated until the land now known as Sand Prairie-Scrub Oak Nature Preserve was purchased in 1969 (McFall and Karnes 1995). When surveyed in 2000 (McClain et al. 2008) this field was dominated by *Eragrostis trichodes* (thread love grass) which had an importance value (I.V.) of 97.4, with *Strophostyles helvula* (wild bean) and *Monarda punctata* (horsemint) ranking second and third in I.V. (possible 200), while *S. scoparium* was absent. When the authors visited this field in the summer of 2008 numerous changes had occurred, the most important being the dominance of *Schizachyrium scoparium* (Table 1). The purpose of the present study was to document these changes in this 39-year-old field, and suggest a probable reason for these changes.

DESCRIPTION OF THE STUDY AREA

Sand Prairie-Scrub Oak Nature Preserve is a 590 ha preserve, located between the villages of Bath and Kilbourne (Sections 13, 14, 23, 26 T20N R9W), Mason County, in the Illinois River Section of the Illinois River and the Mississippi River Sand Areas Natural Division (Schwegman 1973). Sand deposits account for nearly 5% of the land surface of the state, and are common in the northern half of Illinois due to events associated with Wisconsian glaciation (Willman and Frye 1970, Schwegman 1973, King 1981). These sand deposits were formed when glacial lakes drained about 14,500 years ago after glacial moraines were breached. The resulting Kankakee Torrent carried huge amounts of sand and gravel that were deposited when the waters of the Kankakee Torrent slowed upon entering the broad lowlands of the Illinois River below present day Hennepin (Willman 1973). These sands were reworked by wind creating the characteristic dune and swale topography characteristic of these deposits.

Soils of the study site are excessively drained Plainfield sands which contain about 95% sand, are low in organic material, and have a pH of 5.1 to 5.3 (Calsyn 1995). The climate is continental with warm summers and cold winters. Mean annual precipitation is 96.0 cm, with May having the highest rainfall (11.3 cm). Mean annual temperature is 10.8°C with the hottest month being July (24.6°C), and the coldest January (-5.0°C). Frost free days range from 140 to 206 with an average of 173 days (Midwestern Regional Climate Center for Havana, Illinois 2009).

METHODS

Ground-layer species in the 39-year-old field were analyzed in September 2008 using m^2 plots located at 1 m intervals along two 25 m transects oriented east/west in the study area and were located within 5 m of the original transects surveyed in September 2000 (n=25/transect) (McClain et al. 2008). Odd-numbered plots were placed to the right, even-numbered to the left. A random numbers table was used to determine the number of meters (0 to 9) the plot was located from the transect line. Percent cover for each species and bare ground and litter, were determined by using the Daubenmire (1959) cover class system as modified by Bailey and Poulton (1968). The midpoint of each cover class was

used to calculate mean cover, relative cover, frequency (%), relative frequency, and importance value for each species. As used here, IV is the sum of the relative frequency and relative cover. Nomenclature follows Mohlenbrock (2002).

RESULTS

In the 39-year-old field, 27 herbaceous species were present in the 50 plots sampled. Of these taxa *Schizachyrium scoparium* was most important with a frequency of 100%, a mean cover of 50.0%, and an I.V. of 66.8 (Table 1). *Strophostyles helvula* was second with a frequency of 98%, a mean cover of 17.0% and an I.V. of 33.3, while *Physalis heterophylla* (ground cherry), *Leptoloma cognatum* (fall witch grass), *Eragrostis trichodes,* and *Tridens flavus* (purple-top) followed in I.V. In addition to *S. scoparium,* some species that typically dominate mature dry sand prairies were also present, but had low I.V's. The remaining species were all commonly associated with sand prairies, and were mostly present in low numbers, being restricted to the small openings between the bunchgrasses. The only exotic species encountered in the plots was *Mollugo verticillata* (carpetweed) that occurred in very low numbers (Table 1).

DISCUSSION

Mature dry sand prairies in Illinois are dominated by *Schizachyrium scoparium*. This bunch grass commonly formed dense clumps that were 20-60 cm across, and nearly circular in outline. Some of the larger clumps had dead centers in which no other species were found growing. Common associated species of these dry sand prairies were *Ambrosia psilostachya* (western ragweed), *Dichanthelium villosissimum* (hairy panic grass), *Opuntia humifusa* (common prickly pear), and *Tephrosia virginiana* (goat's-rue). In three recent studies of dry sand prairies in Mason County, Illinois these taxa were always present and among the six dominant species of the site (Phillippe et al. 2004, McClain et al. 2005, 2008,).

Although *Schizachyrium scoparium* is a dominant species of mature dry sand prairies in Illinois, it is not found in the early stages of old field succession. At Sand Prairie-Scrub Oak Nature Preserve, McClain and Ebinger (2008) examined the herbaceous vegetation at one-, three-, and twelve-year intervals in a fallow field following clearing of a pine plantation. By the twelfth year the dominant species were *Diodia teres* (I.V. 42), *Eragrostis trichodes* (I.V. 30), *Opuntia humifusa* (I.V. 25), *Aristida desmantha* (I.V. 21), and *Dichanthelium villosissimum* (I.V. 16) while *S. scoparium* was not present.

More recently at Sand Prairie-Scrub Oak Nature Preserve a 60-year-old field abandoned in the early 1940's was surveyed in 2000 (McClain et al. 2008). *Eragrostis trichodes* dominated this field, but it also contained many taxa commonly associated with mature sand prairies with *S. scoparium, Ambrosia psilostachya, D. villosissimum*, and *Opuntia humifusa* being among the top five species found.

In another study at Henry Allan Gleason Nature Preserve similar results were obtained (McClain et al. 2005). This sand prairie was located on the south flank of "Devil's Tower" a large sand dune that dominates Henry Allan Gleason Nature Preserve. Gleason (1910) indicated that Devil's Tower was originally covered with prairie but most had

been destroyed by cultivation and pasturing. The slopes of this dune were planted in pines that were removed in 1978. These slopes presently contain a disturbed dry sand prairie having high species diversity, including some taxa commonly associated with mature dry sand prairies: *Ambrosia psilostachya, Dichanthelium villosissimum, Opuntia humifusa,* and *Schizachyrium scoparium*. *Eragrostis trichoides* was the dominant species with an IV of 47.8 and a mean cover of 16.15.

Some of the dominant species of mature dry sand prairies, such as *Dichanthelium villosissimum* and *Opuntia humifusa,* are present the first year following abandonment (McClain and Ebinger 2008). *Schizachyrium scoparium,* in contrast, does not become an important component until 30 to 40 years after an agricultural field is abandoned. This slow colonization is not due to the lack of a seed source as native prairie adjoins this field to the west and north where *S. scoparium* is the dominant species. It is likely that the formerly cultivated fields lack the mycorrhizal community associated with the roots of *S. scoparium* (Anderson and Liberta 1987, Anderson et al. 1994). The increase in organic material, that is very low in these disturbed soils, may prevent the establishment of the fungus. Slight increases in organic content in these soils over time may allow the establishment of the mycorrhizal community and the corresponding presence of *S. scoparium*.

LITERATURE CITED

Anderson, R.C. and A.E. Liberta. 1987. Variation in vesicular-arbuscular mycorrhizal relationships of two sand prairie species. American Midland Naturalist 127:56-63.

Anderson R.C., B.A.D. Hetrick, and G.W.T.Wilson. 1994. Mycorrhizal dependence of *Andropogon gerardii* and *Schizachyrium scoparium* in two prairie soils. American Midland Naturalist 132:366-376.

Bailey, A.W., and C.E. Poulton. 1968. Plant communities and environmental relationships in a portion of the Tillamook burn, northwestern Oregon. Ecology 49:1-13.

Calsyn, D.E. 1995. Soil survey of Mason County, Illinois. United States Department of Agriculture, Natural Resources Conservation Service, in cooperation with the Illinois Agricultural Experiment Station, Champaign, Illinois.

Daubenmire, R. 1959. A canopy coverage method of vegetation analysis. Northwest Science 33:43-64.

Gleason, H.A. 1910. The vegetation of the inland sand deposits of Illinois. Bulletin of the Illinois State Laboratory of Natural History 9:21-174.

King, J.E. 1981. Late Quaternary vegetational history of Illinois. Ecological Monographs 51:43-62.

McClain, W.E and J.E. Ebinger. 2008. Herbaceous plant succession at Sand Prairie-Scrub Oak Nature Preserve, Mason County, Illinois. Rhodora 110:171-177.

McClain, W.E., L.R. Phillippe, and J.E. Ebinger. 2005. Floristic assessment of the Henry Allan Gleason Nature Preserve, Mason County, Illinois. Castanea 70:146-154.

McClain, W.E., J.E. Schwegman, T.A. Strole, L.R. Phillippe, and J.E. Ebinger. 2008. Floristic study of Sand Prairie-Scrub Oak Nature Preserve, Mason County, Illinois. Castanea 73:29-39.

McFall, D. and J. Karnes. 1995. (editors) A directory of Illinois Nature Preserves, Volume 1 – Northeastern Illinois. Illinois Department of Natural Resources, Springfield, Illinois. 195 pp.

Midwestern Regional Climate Center. 2009. http://mcc.sws.uiuc.edu

Mohlenbrock, R.H. 2002. Vascular Flora of Illinois. Southern Illinois University Press, Carbondale, Illinois. xi+490 pp.

Phillippe, L.R, M.A. Feist, J.E. Ebinger, and W.E. McClain. 2004. Vascular flora of Long Branch Nature Preserve, Mason County, Illinois. Transactions of the Illinois State Academy of Science 97:197-208.

Schwegman, J.E. 1973. Comprehensive plan for the Illinois nature preserves system. Part 2. The natural divisions of Illinois. Illinois Nature Preserves Commission, Rockford, Illinois. map+32 pp.

White, J. and M.H. Madany. 1978. Classification of natural communities in Illinois. Pp. 310-405 in Illinois natural areas inventory. Technical report. (J. White, Editor). Illinois Natural Areas Inventory, Urbana, Illinois.

Willman, H.B. 1973. Geology along the Illinois waterway - a basis for environmental planning. Illinois State Geological Survey Circular 478. Urbana. 48 pp.

Willman, H.B. and J.C. Frye. 1970. Pleistocene stratigraphy of Illinois. Illinois State Geological Survey Bulletin 94:1-204.

Table 1. Frequency (%), mean cover (% of total area), relative frequency, relative cover, and importance value (I.V.) of the ground layer species encountered in the Fall of 2008 in a 39-year-old abandoned field at Sand Prairie-Scrub Oak Nature Preserve, Mason County, Illinois and the I.V. of the species encountered at the same site in a survey conducted in 2000.

	2008					2000
Species	Freq. %	Mean Cover	Rel. Freq.	Rel. Cover	I. V.	I.V.
Schizachyrium scoparium	100	50.00	16.5	50.3	66.8	--
Strophostyles helvula	98	17.00	16.2	17.1	33.3	43.7
Physalis heterophylla	50	6.30	8.3	6.3	14.6	4.8
Leptoloma cognatum	50	4.33	8.3	4.4	12.7	--
Eragrostis trichodes	44	3.76	7.3	3.8	11.1	97.4
Tridens flavus	40	4.22	6.6	4.2	10.8	2.5
Dichanthelium villosissimum	32	3.02	5.3	3.0	8.3	1.2
Ambrosia artemisiifolia	28	2.03	4.6	2.0	6.6	--
Chenopodium simplex	30	0.45	4.9	0.4	5.3	1.1
Eragrostis spectabilis	20	1.81	3.3	1.8	5.1	10.6
Conyza canadensis	24	0.56	4.0	0.6	4.6	1.8
Chamaecrista fasciculata	10	0.62	1.7	0.6	2.3	0.5
Oenothera clelandii	12	0.16	2.0	0.2	2.2	1.1
Asclepias syriaca	8	0.67	1.3	0.7	2.0	2.4
Chrysopsis camporum	6	0.90	1.0	0.9	1.9	0.8
Opuntia humifusa	6	0.90	1.0	0.9	1.9	0.6
Rosa carolina	6	0.66	1.0	0.7	1.7	--
Paspalum bushii	8	0.19	1.3	0.2	1.5	9.8
Solanum carolinense	4	0.60	0.7	0.6	1.3	--
Solidago canadensis	4	0.60	0.7	0.6	1.3	--
Asclepias verticillata	6	0.08	1.0	0.1	1.1	--
Lactuca canadensis	4	0.12	0.7	0.1	0.8	--
Pseudognaphalium obtusifolium	4	0.07	0.7	0.1	0.8	0.6
Croton glandulosus	4	0.02	0.7	--	0.7	--
Solidago nemoralis	2	0.30	0.3	0.3	0.6	--
Teucrium canadense	2	0.06	0.3	0.1	0.4	0.6
Cyperus schweinitzii	2	0.01	0.3	--	0.3	--
Monarda punctata	--	--	--	--	--	16.9
Ampelamus albidus	--	--	--	--	--	1.1
Brickellia eupatorioides	--	--	--	--	--	0.9
Dichanthelium oligosanthes	--	--	--	--	--	0.6
Ipomoea lacunosa	--	--	--	--	--	0.5
Mollugo verticillata	--	--	--	--	--	0.5
Totals		99.44	100.0	100.0	200.0	200.0
Bare ground and litter		16.50				

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Loss of Hulsebus Loess Hill Prairie, Henderson County, Illinois to Woody Plant Invasion

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ABSTRACT

Hulsebus Hill Prairie, Henderson County, Illinois, was recognized by the Illinois Natural Areas Inventory in 1976 as a good quality loess hill prairie. The dominant herbaceous plants were *Bouteloua curtipendula* (sideoats grama), *Schizachyrium scoparium* (little bluestem), *Asclepias verticillata* (whorled milkweed), and *Dalea candida* (white prairie clover). Twenty-one herbaceous prairie species were present in the 0.2 ha prairie. The prairie was overgrown with woody vegetation by 1997 and A*geratina altissima* (white snakeroot) had become the dominant herbaceous taxon on the hillside. A 2008 study showed an increase in herbaceous vegetation associated with forest communities. A dense cover of *Cornus drummondii* (rough-leaved dogwood) covered the site and no hill prairie vegetation remained.

INTRODUCTION

Loess hill prairies are prominent features of the Illinois, Mississippi, and Sangamon river bluffs. Due to their presence on steep terrain, they were not converted to row crop agriculture like loam soil prairies. Some hill prairies are currently regarded among the highest quality prairie remnants in Illinois. Despite these features, few studies were completed on hill prairies in Illinois during the early 1900s. The classic publication "Hill Prairies of Illinois" by R. A. Evers (1955) brought widespread recognition to these hillside grasslands.

Dr. Robert Evers observed the effects of woody invasion on some hill prairies following his initial study and began accumulating data for a second publication, but was unable to complete the work. Other authors described woody invasion in several parts of Illinois

(Kilburn and Warren 1963, Reeves et al 1978, Ebinger 1981, Behnke and Ebinger 1989). McClain (1983) documented the loss of sixty-two percent of the area of five hill prairies at Pere Marquette State Park, Jersey County, Illinois. Other studies described widespread fragmentation of hill prairies and made predictions that small unmanaged hill prairies would disappear within 20 years (McClain and Anderson 1990, Robertson et al. 1995, Schwartz et al. 1997).

One of the primary woody invaders of hill prairies in Illinois is *Cornus drummondii* C. A. Mey. (rough-leaved dogwood). This shrub is known for the formation of dense thickets that encircle and advance upon hill prairie communities. These dense shrub zones create shade and competition detrimental to prairie species, thus initiating the conversion from prairie to forest (McClain and Anderson 1990).

The Illinois Natural Areas Inventory (INAI) conducted a statewide survey in the 1970s to identify high quality remnants of natural vegetation (White 1978). Six hill prairies were recognized in Henderson County, including a small loess hill prairie, known as Hulsebus Hill Prairie, located on the Mississippi River bluffs near Olena. The dominant herbaceous plants present on this site were *Bouteloua curtipendula* (sideoats grama) *Schizachyrium scoparium* (little bluestem), *Asclepias verticillata* (whorled milkweed), and *Dalea candida* (white prairie clover). Twenty-one herbaceous prairie species were observed on the prairie (Table 1).

In 1993, while conducting surveillance of natural areas, Illinois Nature Preserves Commission (INPC) and the Illinois Department of Natural Resources (IDNR) personnel described Hulsebus Hill prairie as "barely hanging on" due to woody plant invasion. An INPC representative was unable to find any trace of the hill prairie in 1996. Field studies in 1997 and 2008 confirmed the location and documented the disappearance of the hill prairie. The purpose of this study was to document the transformation of Hulsebus Hill Prairie to forest and to describe factors causing its disappearance.

DESCRIPTION OF THE STUDY AREA

Hulsebus Hill Prairie is located in Stronghurst Township (T 9N, R5W, SW ¼ SE ¼ SE ¼ of S5), Henderson County in northwestern Illinois approximately four km northwest of the Village of Olena and ten km northwest of the Village of Stronghurst. Ellison Creek, a permanent stream, is less than a km from the site, and the Mississippi River lies approximately five km to the west.

Hickory silt loam is the common soil of the bluffs and slopes of Henderson County. Hickory soils are very deep, well drained, and highly dissected. The parent material of this soil is glacial till capped with up to 20 inches of wind-blown loess. The upper horizons contain rock fragments, up to 20 percent sand, and are strongly acidic (Heisner and Elmer 2006).

The climate of west-central Illinois is continental, characterized by hot, humid summers and cold winters. The highest recorded temperature at Aledo in nearby Mercer County is 39 C in September of 1953, while the lowest temperature of -27 C was recorded on January 17, 1977. The average annual precipitation of 88.5 cm falls mostly as rain during

April to September. The average annual snowfall is 82.3 cm. During an average year, 49 days will have 2.5 cm or more of snow on the ground (Heisner and Elmer 2006).

METHODS

Field data were obtained from the 1976 INAI survey of Hulsebus Loess Hill Prairie. The INAI ecologists utilized 30 1/4m circular plots to determine frequency of hill prairie vegetation. The 1997 survey utilized 1/4 m square plots to study herbaceous plants and woody seedlings less than 1m tall along two randomly located north/south transects, each 25 m in length (50 quadrants). The 2008 study utilized one m^2 quadrants placed along two 25 m transects oriented east to west across the hillside in the area formerly occupied by prairie vegetation (50 quadrants). Quadrants were placed at one m intervals along the transects with even-numbered plots being placed to the right and odd-numbered plots to the left. Cover for each species was determined using the procedure described by Daubenmire (1959) as modified by Bailey and Poulton (1969). From this data relative frequency, relative cover, and Importance Value were determined.

Woody vegetation was studied along two line transects, each 2 m x 25 m, that were placed east/ west across the hillside. All small saplings (over 1 m in height but less than 1 cm dbh) and large saplings (over 1 m in height and greater than 1 cm dbh) were counted. These data were used to estimate stems/ha for each species. Nomenclature follows Mohlenbrock (2002).

RESULTS

The INAI field ecologists documented the presence of a high quality loess hill prairie community, 0.16 ha in size, at this site on August 16, 1976. The hill prairie community was described as having a good grass cover, but low wildflower diversity. Herbaceous prairie species had disappeared when McClain and Moorehouse surveyed the site on October 7, 1997. Species associated with woodlands, including *Ageratina altissima* (white snakeroot) and the woody vine *Toxicodendron radicans* (poison ivy) were dominant. The only prairie species located were two plants of side oats grama and five plants of little bluestem (Table 1).

White snake root was the dominant plant in the herbaceous understory with an IV of 29.4 when the site was visited on September 21, 2008. Other prominent understory plants were *Euonymus atropurpureus* (wahoo), *Toxicodendron radicans* (poison ivy), *Viola sororia* (downy blue violet), and *Parthenocissus quinquefolia* (Virginia creeper). Several herbaceous plants and woody vines, commonly regarded as forest herbs, were more prominent in 2008, including *Aquilegia canadensis* (columbine), *Solidago ulmifolia* (elm-leaved goldenrod), *Sanicula odorata* (common black snakeroot), *Smilax tamnoides* (greenbriar) and *Menispermum canadense* (moonseed) (Table 1).

The woody vegetation is currently dominated by *Cornus drummondii* (rough-leaved dogwood), *Ulmus americana* (American elm), and *Celtis occidentalis* (hackberry). The density of dogwood, including small and large saplings, exceeded 12,000 individuals per ha. The invasive exotic shrub, *Lonicera maackii* (Amur honeysuckle), was common (Table 2).

DISCUSSION

All hill prairies, regardless of size or landscape position, are susceptible to invasion and conversion to forest by woody plants. Small hill prairies located within densely forested lands are especially vulnerable, as evidenced by Hulsebus Hill Prairie. The amount of time required for the conversion from hill prairie to forest has been estimated to be a maximum of 15 to 20 years (McClain and Anderson 1990). Hulsebus Hill Prairie was overwhelmed with woody vegetation 17 years after its discovery.

One of the primary invaders of hill prairies is rough-leaved dogwood, a species known for its high seed production and prolific sprouting abilities. This taxon produces dense, nearly pure stands that frequently encircle and advance upon hill prairie communities. The extensive root systems of rough-leaved dogwood are difficult to control, and prescribed fires often fail to impact the dense growth of shrubs due to a reduced biomass of prairie grasses. Stopping woody invasion is not an easy task. Even intensively managed hill prairies do not show a reversal of woody invasion or an expansion of hill prairie vegetation (Robertson et al.1995). Managers trying to duplicate landscape fires on small parcels of land will find the task of maintaining prairie communities increasingly difficult or impossible due to urbanization and other restrictions.

Invasive exotic woody species, primarily Amur honeysuckle, also contribute to the loss of hill prairie. Individuals of this species were present at Hulsebus (Table 2) and at Pere Marquette in Jersey County (McClain and Anderson 1990). Glacial drift hill prairies near Charleston in Coles County contain high densities of *Euonymus alatus* (burning bush) (Behnke and Ebinger 1989), and field ecologists have observed high densities of *Albizia julibrissin* (mimosa) in loess hill prairies of Randolph County in southwestern Illinois (Debbie Neumann, personal observation).

Hill prairie reconstruction has also been greatly neglected. Except for Kilburn and Warren (1963), no efforts have been made to recreate these communities on former hill prairie sites. This contrasts with the extensive reconstruction of mesic black-soil prairies over the last 50 years (McClain 2003, Kurtz 2001, Schramm 1976, 1990). Quantitative studies of the flora and fauna of hill prairies will be critical templates in reconstruction efforts in the future.

The profound changes taking place in hill prairies are considered permanent. Many studies have documented the replacement of hill prairie vegetation by shrubs and small trees (Behnke and Ebinger 1989, McClain 1983, Robertson et al. 1995, Schwartz et al. 1997). Woody invasion of hill prairies will be a continuing problem for decades and many hill prairies will be converted to forest. The disappearance of two other Henderson County Hill Prairies, Bailey Hill Prairie and Delabar-Pruitt Hill Prairie, was recently confirmed by INPC representative Angella Moorehouse, leaving only three of the original six INAI hill prairies intact.

LITERATURE CITED

Bailey, W. W., and C. E. Poulton. 1969. Plant communities and environmental relationships in a portion of the Tillamook burn, northwestern Oregon. Ecology 49:1-13.

Behnke, G., and J. E. Ebinger. 1989. Woody invasion of glacial drift hill prairies in east-central Illinois. Transactions of the Illinois State Academy of Science 82:1-4.

Daubenmire, R. 1959. A canopy coverage method of vegetation analysis. Northwest Science 33: 43-64.

Ebinger, J. E. 1981. Vegetation of glacial drift hill prairies in east-central Illinois. Castanea 46:115-121

Evers, R. A. 1955. Hill prairies of Illinois. Illinois Natural History Survey Bulletin 26:367-446.

Heisner, F. E. and S. L. Elmer. 2006. Soil Survey of Henderson County, Illinois. Soil Conservation Service, United States Department of Agriculture. Washington, D. C.

Kilburn, P. D., and D. K. Warren. 1963. Vegetation-soil relationships in hill prairies. Transactions of the Illinois State Academy of Science 56:142-145.

Kurtz, C. 2001. A Practical Guide to Prairie Reconstruction. University of Iowa Press. Iowa City. 57 pp.

McClain, W. E. 1983. Photodocumentation of the loss of hill prairie within Pere Marquette State Park, Jersey County, Illinois. Transactions of the Illinois State Academy of Science 76:343-346.

McClain, W. E. 2003. Prairie Establishment and Landscaping, Technical Publication #2, Revised Edition. Division of Natural Heritage. Illinois Department of Natural Resources. Springfield, IL 66 pp.

McClain, W. E., and E. A. Anderson. 1990. Loss of hill prairie through woody plant invasion at Pere Marquette State Park, Jersey County, Illinois. Natural Areas Journal 10:69-75.

Mohlenbrock, R. H. 2002. Vascular Flora of Illinois. Southern Illinois University Press. Carbondale. 490 pp.

Reeves, J. T., U. D. Zimmerman, and J. E. Ebinger. 1978. Microclimatic and soil differences between hill prairies and adjacent forests in East-Central Illinois. Transactions of the Illinois State Academy of Science 71: 156-164.

Robertson, K. R., M. W. Schwartz, J. W. Olson, B. K. Dunphy, and H. D. Clarke. 1995. Fifty years of change in Illinois Hill Prairies. Erigenia 14:41-52.

Schramm, P. 1976. The "do's and don'ts of prairie restoration." Pages 138-150 in Glenn-Lewis, D. C., and R. Q. Landers, Editors. Proceedings of the Fifth Midwest Prairie Conference. Iowa State University, Ames, Iowa. 230 pp.

Schramm, P. 1990. Prairie restoration: A twenty-five year perspective on establishment and management. Pages 169-178 in Smith, D. D., and C. A. Jacobs, Editors. Recapturing a vanishing heritage. Proceedings of the Twelfth North American Prairie Conference. University of Northern Iowa. Cedar Falls, IA. 2318 pp.

Schwartz, M. W., K. R. Robertson, B. K. Dunphy, J. W. Olson, and A. M. Trame. 1997. The biogeography of and habitat loss on hill prairies. Pages .267-283. in M. M. Schwartz, ed., Conservation of Highly Fragmented Landscapes. Chapman & Hall. New York.

White, J. 1978. Illinois Natural Areas Inventory Technical Report. Illinois Natural Areas Inventory, Urbana. 426 pp.

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Table 1. Frequency (F), relative frequency (RF), relative cover (RC), and importance values (IV) for ground layer vegetation in 1976, 1997, and 2008 at Hulsebus Hill Prairie, Henderson County, Illinois.

	1976		1997				2008			
Species	F	RF	F	RF	RC	IV	F	RF	RC	IV
Bouteloua curtipendula	97.0	23.2	2	0.5	0.6	1.1	--	--	--	--
Schizachyrium scoparium	90.0	21.6	4	1.0	0.2	1.2	--	--	--	--
Asclepias verticillata	40.0	9.6	--	--	--	--	--	--	--	--
Dalea candida	40.0	9.6	--	--	--	--	--	--	--	--
Dichanthelium sp.	30.0	7.2	16	3.9	0.6	4.5	--	--	--	--
Melilotus albus	27.0	6.4	18	4.4	6.1	10.5	--	--	--	--
Lithospermum incisum	23.3	5.6	--	--	--	--	--	--	--	--
Ruellia humilis	20.0	4.8	8	2.0	1.5	3.5	--	--	--	--
Aster sp.	10.0	2.4	4	1.0	1.0	2.0	--	--	--	--
Poa pratensis	6.7	1.6	12	3.0	1.0	4.0	--	--	--	--
Amorpha canescens	6.7	1.6	--	--	--	--	--	--	--	--
Cornus drummondii	6.7	1.6	14	3.4	5.1	8.5	18	3.3	4.4	7.7
Chamaecrista fasciculata	3.3	0.8	--	--	--	--	--	--	--	--
Pycnanthemum tenuifolium	3.3	0.8	--	--	--	--	--	--	--	--
Bouteloua hirsuta	3.3	0.8	--	--	--	--	--	--	--	--
Solidago nemoralis	3.3	0.8	--	--	--	--	--	--	--	--
Prunus serotina	3.3	0.8	--	--	--	--	6	1.0	0.4	1.4
Toxicodendron radicans	--	--	48	11.8	23.4	35.2	32	5.9	8.5	14.4
Ageratina altissima	--	--	52	12.8	16.0	28.8	72	13.3	16.1	29.4
Carex pensylvanica	--	--	36	8.9	6.6	15.5	64	11.9	6.2	18.1
Carex sp.	--	--	34	8.4	2.1	10.5	--	--	--	--
Parthenocissus quinquefolia	--	--	18	4.4	6.0	10.4	28	5.2	6.6	11.8
Solidago ulmifolia	--	--	12	3.0	6.1	9.1	20	3.6	6.1	9.7
Smilax tamnoides	--	--	18	4.4	4.0	8.4	24	4.5	5.0	9.5
Quercus rubra	--	--	12	3.0	5.2	8.2	16	3.0	3.2	6.2
Muhlenbergia sobolifera	--	--	10	2.5	3.0	5.5	2	0.4	0.1	0.5
Celtis occidentalis	--	--	10	2.5	1.4	3.9	26	4.8	5.0	9.8
Viola sororia	--	--	10	2.5	1.3	3.8	46	8.5	2.0	10.5
Celastrus scandens	--	--	8	2.0	1.4	3.4	12	2.0	3.0	5.0
Galium circaezans	--	--	4	1.0	1.1	2.1	4	0.7	0.2	0.9
Lactuca canadensis	--	--	4	1.0	1.1	2.1	--	--	--	--
Euonymus atropurpureus	--	--	4	1.0	1.1	2.1	38	7.0	8.3	15.3
Aquilegia canadensis	--	--	4	1.0	1.0	2.0	34	6.3	1.5	7.8
Fraxinus americana	--	--	6	1.5	0.2	1.7	--	--	--	--
Xanthoxylum americanum	--	--	4	1.0	0.6	1.6	--	--	--	--
Vitis vulpina	--	--	--	--	--	--	10	1.9	3.3	5.2
Veronicastrum virginicum	--	--	--	--	--	--	8	1.5	3.7	5.2
Elymus virginicus	--	--	--	--	--	--	14	2.6	2.0	4.6
Menispermum canadense	--	--	--	--	--	--	8	1.5	2.6	4.1
Sanicula odoratas	--	--	--	--	--	--	10	1.9	2.1	4.0
Campanulastrum americanum	--	--	--	--	--	--	10	1.9	1.6	3.5
Desmodium glutinosum	--	--	--	--	--	--	4	0.7	1.3	2.0
Helianthus divaricatus	--	--	--	--	--	--	4	0.7	1.3	2.0
Juniperus virginiana	--	--	--	--	--	--	4	0.7	1.0	1.7
Lonicera maackii	--	--	--	--	--	--	4	0.7	1.0	1.7
Symphorocarpos orbiculatus	--	--	--	--	--	--	4	0.7	1.0	1.7
Ulmus americana	--	--	--	--	--	--	4	0.7	1.0	1.7
Carya ovata	--	--	--	--	--	--	4	0.7	0.3	1.0
Others	--	--	--	8.1	2.3	10.4	--	2.4	1.2	3.6
Totals	--	99.2	--	100.0	100.0	200.0	--	100.0	100.0	200.0

Table 2. Density (stems/ha) of the small (>50 cm tall, ≤2.5 cm dbh) and large saplings (2.6-5.0 cm dbh) encountered at Hulsebus Loess Hill Prairie, Henderson County, Illinois.

Species	Small Saplings	Large Saplings
Cornus drummondii (rough-leaved dogwood)	10400	2200
Toxicodendron radicans (poison ivy)	900	--
Ulmus americana (American elm)	500	300
Celtis occidentalis (hackberry)	500	100
Ostrya virginiana (hop hornbeam)	100	100
**Lonicera maackii* (Amur honeysuckle)	100	--
Quercus rubra (red oak)	100	--
Juniperus virginiana (red cedar)	--	100
Totals	12600	2800

* = Non-native species

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ISSR Marker Diversity of the Threatened *Astragalus crassicarpus* var. *trichocalyx* in Illinois Populations

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ABSTRACT

Astragalus crassicarpus Nutt. var. *trichocalyx* (Nutt.) Barneby is a perennial plant that occurs in the southeastern part of the Great Plains on prairies, open oak woodlands, limestone hills and riverbanks. The species was considered extirpated from Illinois until it was rediscovered in 1987 on a limestone bluff in Beaver Dam State Park in Jersey County. It is now on the state list of endangered plant species. Presently three populations exist in Macoupin County, Illinois within isolated glacial till prairie sites. In Illinois *A. crassicarpus* populations are small and restricted to areas within the prairie sites. The three existing populations are separated by geographical barriers, which severely restricts gene flow or seed dispersal between populations. The sites themselves are fragile due to the encroachment of woody species. All of these factors increase the threat of extinction for the species in Illinois. Conversely, *A. crassicarpus* exists in several large populations in Missouri such as Painted Rock Conservation Area in Osage County. If conservation strategies are to be developed, an understanding of the genetic variation of the species in large and endangered populations is essential. One molecular marker that has proven useful in genetic diversity studies are ISSR markers. We compared the large population in Missouri to the small endangered populations in Illinois using ISSR markers. Painted Rock Conservation Area had the highest within population diversity value (0.574) of all sampled populations. On average, the Illinois populations had a lower diversity value than the Missouri population (0.482). Interestingly, Roderick Prairie, the smallest population in Illinois, had a diversity value of 0.525. Every Illinois population sampled contained unique genetic variation not found in the Missouri population. Because of this diversity and how it is partitioned, it is important to preserve as many Illinois populations as possible, large or small.

INTRODUCTION

Astragalus crassicarpus Nutt. var. *trichocalyx* (Nutt.) Barneby is a perennial plant that occurs in the southeastern part of the Great Plains on prairies, open oak woodlands, limestone hills and riverbanks (McGregor 1986). It is a member of the Fabaceae family and is commonly known as prairie groundplum or milkvetch (USDA 2007). It is native to North America and ranges from Wisconsin to Texas (USDA 2007). *Astragalus crassicarpus* var. *trichocalyx* is endangered in Illinois and Wisconsin (USDA 2007).

The species was considered extirpated from Illinois until it was rediscovered in 1987 on a limestone bluff in Beaver Dam State Park in Jersey County, and it is now on the state list of endangered plant species (Herkert and Ebinger 2002). *Astragalus crassicarpus* var. *trichocalyx* occurs in three localities in the state, all of them on glacial drift prairies in Macoupin County (McClain et al. 2002). *Astragalus crassicarpus* exists in several large populations in Missouri such as Painted Rock Conservation Area in Osage County (USDA 2007).

There are five varieties of *A. crassicarpus* (USDA 2007). Variety *berlandieri* occurs in Texas, and variety *cavus* occurs in New Mexico and Arizona (USDA 2007). Varieties *crassicarpus* and *paysonii* are widespread from Montana to Texas (USDA 2007). The variety examined in this study is *trichocalyx*, which occurs in seven states from Illinois south and west to Texas (USDA 2007).

The genus *Astragalus* contains two other species which are listed as endangered in Illinois (INHD 2007). *Astragalus distortus*, also known as Ozark milkvetch, occurs in four counties (INHD 2007). *Astragalus tennesseensis,* or Tennessee milkvetch, is currently found in two counties (INHD 2007).

Illinois contains many prairie inclusions, but glacial drift prairies are not common throughout the state (McClain et al. 2003). They are sometimes called gravel, dolomite, or glacial till prairies (IDNR 2007). Glacial drift prairies contain sediment deposited from the last ice age (McClain et al. 2003). In Illinois at the end of the Pleistocene, 126,000 years ago to 5,000 years ago, the glacier masses retreated to leave what we now know as glacial drift prairies (IDNR 2007). Glacial drift prairies are dry, well drained, and slightly alkaline (McClain et al. 2003). Many in Illinois have been destroyed by modern development and agriculture (IDNR 2007).

Glacial drift prairies tend to occur on slightly rolling, rocky terrain (McClain et al. 2003). Fires and nutrient-poor soil helped to keep these prairie areas free from invasion by woody species in pre-settlement times (Ebinger and McClain 1991). Their range in Illinois, however, has decreased because of fire suppression by European settlers (McClain et al. 2002). Glacial drift prairie remnants are rare and small (less than one hectare in size) and, therefore, have not received as much attention as other communities in the state (McClain et al. 2002). There is no known reason for the restriction of *A. crassicarpus* to glacial till prairies in Illinois.

Other species are restricted to glacial drift prairies in Illinois as well. *Lespedeza leptostachya* or prairies bush clover, *Dalea foliosa* or leafy prairie clover, and *Hymenoxys acaulis* var. *glabra* or lakeside daisy are all endangered in Illinois (USFW 2007). The natural populations of lakeside daisy were extirpated when a mining company destroyed its site (USFW 2007). Preserving the glacial till prairie habitats of Illinois will help conserve not only *A. crassicarpus* var. *trichocalyx*, but all of the species mentioned above.

In a 2006 thesis by Wells, some *A.crassicarpus* var. *trichocalyx* life history characteristics are presented. All of these following factors may contribute to its

endangerment in Illinois. Soil cores were taken in all three Illinois prairies to assess the seed bank (Rogenski et al. 2004). The soil seed bank is small to nonexistent (Rogenski et al. 2004). Seed scarification experiments were performed on *A. crassicarpus* seeds (Wells 2006). Scarification is rupturing of the seed coat by scratching or softening to encourage germination and is required for germination of *A. crassicarpus* seeds (Baskin & Quaterman, 1969). Disturbance, such as burnings, may aid in scarification and thus germination. If burnings are not done annually, other species or invasive species may out compete *A. crassicarpus*. Other members of the genus are buzz pollinated (Kearns et al. 1998). *Astragalus crassicarpus* var. *trichocalyx* is most likely buzz pollinated by *Bombus perplexus*, which requires the insect to grab onto the flower and move their flight muscles which causes the flower and anthers to vibrate, releasing the pollen. This vibration is called buzz pollination. It is not known if it is self incompatible, because self incompatibility varies in the genus. *Astragalus crassicarpus* does not asexually reproduce (Reveal 1978). If there are not enough compatible mating types available there may be reduced seed production, and population size could even further decrease.

Understanding genetic variation is essential to the population structure and rarity of *A. crassicarpus* var. *trichocalyx*. The more diversity within a taxon, the better it can deal with evolutionary and environmental changes (Godt et al. 1996). In order to safeguard the Illinois sites, genetic understanding of the species in both large and endangered populations is key. Response of a species to environmental changes, disease resistance, and climatic conditions depends on the amount of genetic variation within a population (Godt et al. 1996).

One way to estimate the amount of genetic diversity in populations is through the use of molecular markers. Molecular markers are DNA markers that assess variation based on differences in the DNA sequence. One molecular marker that has proven useful in genetic diversity studies is ISSR (inter-simple sequence repeats) markers (Esselman et al. 1999; Alexander et al. 2004). Unlike other molecular markers, ISSRs require no prior knowledge of the exact DNA sequence (Godwin et al. 1997). Also, ISSRs show more genetic diversity than other molecular markers and have been used to examine genetic diversity of imperiled species (Nagaoka and Ogihara 1997; Esselman et al. 1999; McGlaughlin 2002; Alexander et al. 2004). They require less time, money, and labor than other methods and exhibit Mendelian inheritance (Harris 1999; Alexander et al. 2004). The technique behind ISSR markers makes use of PCR (polymerase chain reaction) and ISSR primers. The primers bind to sequence repeats in the genome, these regions get amplified via the PCR reaction and create DNA fragments of various sizes. The fragments produced are separated by size on an agarose gel by passing a current through the gel, a process known as gel electrophoresis. The Missouri population was chosen because of the large number of individuals and relatively close proximity to Edwardsville, Illinois. Permission was given by Tim Smith of the Missouri Department of Conservation to collect individuals from this conservation area.

We hypothesized that the isolated populations of *Astragalus crassicarpus* var. *trichocalyx* in Illinois will have lower levels of ISSR marker diversity than a larger population in Missouri. Loss of habitat, smaller populations, and restriction of Illinois populations to glacial drift prairies all predict lower diversity (Godt et al. 1996). Because of geographical barriers between populations, human interference, such as cattle grazing and

restriction to glacial drift prairies, the Illinois populations should contain lower levels of diversity. This lack of diversity will affect the way *A. crassicarpus* responds to environmental and evolutionary change (Godt et al. 1996). *Astragalus crassicarpus* exists in 54 counties throughout the state of Missouri (USDA 2007). The population at Painted Rock Conservation Area is large and should contain more ISSR diversity because it is less restricted by glacial till prairies, endemic habitats, and small population size.

MATERIALS AND METHODS

Study Sites

Plants were collected in 2005 from Beaver Dam State Park, Humphries Prairie, and Roderick Prairie Nature Preserve, all of which occur within 16 km of each other on glacial till prairies in Macoupin County Illinois (Figure 1). Individuals were also collected in 2005 from Painted Rock Conservation Area located in Osage County Missouri, approximately 225 km from Edwardsville, Illinois. With thousands of individuals, it is a very large population, and will provide a genetic comparison to the much smaller endemic populations of Illinois.

Figure 1. Location of sites: Macoupin County, Illinois and Osage County, Missouri.

Beaver Dam State Park is the largest of the Illinois populations with 278 individuals in 2004 (Wells 2006). The site is divided into two sections by a four-wheel track. It has been subjected to burns approximately every two to three years in spring (Mark Phipps, Illinois Department of Natural Resources, personal communication with Wells 2006).

Humphries Prairie is located on private land and from 1999-2000 was grazed by cattle (Wells 2006). Cattle grazing may continue, but it is not documented. In 2004, Humphries Prairie had a total of 167 individuals (Wells 2006).

Roderick Prairie Nature Preserve is the smallest of the Illinois populations. This site is also located on private land and is partially bordered by agricultural land. In 2004, the population consisted of 67 individuals (Wells 2006).

Method of Collection

Samples were collected in mid to late summer of 2005 along transects at each site. Samples from Beaver Dam State Park, Humphries Prairie, and Roderick Nature Preserve were collected on the same day. Collection from Painted Rock Conservation Area occurred at a later date in summer of 2005. During collection the leaf tissue was placed in a small bag with silica gel and numbered. Samples consisted of leaf tissue, where younger tissue was preferred to older tissue.

DNA Extraction and Isolation

A DNeasy Qiagen kit and protocol was used in 2007 to isolate the DNA from dried material. Approximately 0.04 mg of leaf tissue was used in each extraction. Because of potential DNA degradation, the gels were run within one week of isolation.

PCR Procedures

Three primers were utilized in this study: 17898, 17899, and 17901 (Esselman et al. 1999). All were obtained from GenoSys. Reactions were carried out in a volume of 23 mL consisting of 3 mL $MgCl_2$, 2 mM dNTPs, 1 mL primer, a volume of DNA, 0.7 mL Taq DNA polymerase and 2.5 mL x Taq DNA polymerase buffer. A Strategene Robocycler was used with the thermocycle program set at 1.5 min at 94°C, 35 x 40 s at 94°C, 45 s at the annealing temperature, 1.5 min at 72°C; 45 s at 94°C, 5 min at 72°C, 6°C soak. The primer designations and compositions were 17898 $(CA)_6RY$, 17899$(CA)_6RG$, and 17901$(GT)_6YR$.

ISSR Analysis

The gels were run at 100 V (approximately 65 m amps) until the marker dye moved a total of 7 cm. The PCR generated ISSR markers were separated out on 1.4% agarose gel in 1X TBE Buffer. The gels were stained with ethidium bromide. A 100 bp ladder (Gibco/BRL) was run with the PCR generated fragments to determine sizes of the fragments. The gels were read and analyzed using the Bio-Rad Gel Doc XR Molecular Imager. The banding patterns for each gel were read as present (1) or absent (0). Only band presence values were entered into a program written by Vera Ford, UC-Davis, revised by Timothy York, to generate average pairwise similarity values (Esselman et al. 1999).

The similarity values were recorded and then entered into NTSYSpc Version 2.02 (Rohlf 1998) for UPGMA (unweighted pair group using arithmetic average) cluster analysis and to generate the phenogram. The similarity and clustering methods of analysis were done to determine the amount of variation within and among populations. The number of unique bands and the percent of variable bands were tabulated for each population.

RESULTS

A total of 50 bands were scored. Primer 17898 had a total of 22 bands. Primer 17899 had a total of 17 bands. Eleven bands were scored for primer 17901.

Table 1. Distance values for Missouri and Illinois populations. Within population distances values are italicized.

	Missouri	Beaver Dam	Humphries	Roderick
Missouri	*0.574*			
Beaver Dam	0.715	*0.491*		
Humphries	0.712	0.659	*0.429*	
Roderick	0.692	0.627	0.647	*0.525*

Table 1 indicates the distance values for all populations analyzed using ISSR markers. A distance value of one indicates complete ISSR marker similarity among compared individuals. Between the Illinois populations, Humphries has the lowest within population diversity (0.429). Beaver Dam Prairie has a distance value of 0.491, slightly higher than Humphries Prairie. Roderick has a distance value of 0.525. These values indicate slightly lower levels of diversity than the largest population, Painted Rock Conservation Area in Missouri. The Missouri population has the highest within population distance value (0.574). In comparisons between populations (Table 1, Figure 2), the three Illinois populations are more similar to one another then either is to the Missouri population.

Table 2. Number of individuals sampled, unique bands, and percent unique bands.

Population	Number of Individuals	Number of Unique Bands	% Unique Bands
Missouri	16	7	14
Beaver Dam	22	2	4
Humphries	13	1	2
Roderick	18	3	6

There were unique ISSR marker bands (Table 2) in every population. Missouri, with seven, had the most unique bands (14%). Roderick, the smallest Illinois population, had a total of three unique bands (6%). Beaver Dam, the largest Illinois population, had a total of two unique bands (4%). Humphries Prairie contained only one unique band (2%). All unique band percentages of the Illinois populations were lower than the Missouri population.

DISCUSSIONS, CONCLUSIONS, RECOMMENDATIONS

Astragalus crassicarpus populations in Illinois are small, with fewer than 400 plants (Wells 2006). Obstacles such as roads, fields, farms, and bodies of water severely restrict gene flow or seed dispersal between populations. Dispersal by larger animals from one prairie to another may not be realistic because of these geographical barriers. The sites themselves are fragile due to the encroachment of woody species (Wells 2006), and all sites occur within a small geographic range, referred to as endemism by conservation biologists (Anderson 1994). All of these factors increase the threat of extirpation for the species in Illinois (Anderson 1994).

Based on the results of this ISSR study, every measure should be taken to protect *A. crassicarpus* populations in Illinois both large and small. Painted Rock Conservation Area had a distance value of 0.574 (Table 1). The average distance value of the Illinois populations was 0.482. This supports our hypothesis that there is more ISSR marker diversity in the larger population of Missouri as compared to the endangered, endemic populations of Illinois.

In Illinois we found that population size did not correlate with high levels of diversity. The largest population in Illinois, Beaver Dam State Park, had an intermediate distance value of 0.491. Although this site contains more individuals, it is in close contact to human traffic including four wheelers and grazing cattle. This disturbance may affect flowering, seedling survival, or seed dispersal and results in lower diversity values. Roderick, the smallest population, had the highest diversity value (0.525). Because the smaller Illinois populations have high levels of diversity, every existing population needs to be preserved.

In comparisons between populations, the three Illinois sites are more similar to each other than any of them are to the Missouri population (Table 1, Figure 2). All populations sampled contained unique bands (Table 2). Unique bands are those that only exist in one population and are a measure of unique genetic information. As we expected, the largest population sampled, Missouri, had the most unique markers (7). Illinois had an average of two unique bands per population. Roderick, the smallest Illinois population, had a total of three unique bands (6%).

The percent unique bands would be expected to decrease as population size decreases. This was not supported as Roderick, the smallest population, contained the most unique bands of all Illinois populations. This is important because even the smallest Illinois population has the most unique genetic information which could be important to the survival of the species in the state.

Similar to *A. crassicarpus* var. *trichocalyx*, *Trifolium stoloniferum* or running buffalo clover is a member of the Fabaceae family (Crawford et al. 1998). It is restricted to five states and is currently federally endangered (Crawford et al. 1998). Crawford et al. (1998) sampled 390 individuals from 21 populations. The average distance value of *T. stoloniferum* is 0.048 while the average distance value of *A. crassicarpus* in Illinois populations is 0.482 (Crawford et al. 1998). This suggests that *A. crassicarpus* has

maintained a high level of diversity in its small Illinois populations compared to another member of the legume family.

Karron et al. (1988) investigated the population genetic structure of two locally endemic *Astragalus* species with enzyme markers. He found that these restricted populations were not genetically depauperate. Karron et al. (1988) suggests that diversity in these endemic populations is the result of changes in distribution ranges. *Astragalus crassicarpus* is widespread from Wisconsin to Texas, but the species is endemic in Illinois. This suggests that the remaining Illinois populations and the diversity they contain are remnants of a once larger distribution range. At this time, there are no historical records of the species distribution in Illinois.

Future research on *A. crassicarpus* var. *trichocalyx* should involve genetic comparisons of endangered Illinois populations with successful populations in more states, including other habitats similar to glacial drift prairies. Another study that should be undertaken is to determine if *A. crassicarpus* is self-incompatible. Understanding the reproductive biology of this species is crucial to developing management plans.

With over 99.9% prairie habitat loss in Illinois, *Astragalus crassicarpus* var. *trichocalyx* is a remaining representative of this once abundant ecosystem (IDNR 2007). *Astragalus crassicarpus* has maintained ISSR diversity in the endangered Illinois populations even with limited habitat and individuals. This diversity gives hope to reviving the populations.

Future conservation efforts may include establishing more plants in the Illinois populations and maintaining the habitats with consistent burnings. Other members of the genus *Astragalus*, *A. purshii* and *A. oniciformis,* have been successfully reintroduced into areas by seeding or by transplanting (Wirth and Pyke 2003; Popovich and Pyke 1997). Seedling survival and reduction in the age to first flowering are enhanced by burning in *A. purshii* (Wirth and Pyke 2003). These factors may promote population fitness and increase population sizes over time (Wirth and Pyke 2003). Experimentation with prescribed burns and re-establishing plants in the Illinois sites could successfully increase plant population numbers and survival over time.

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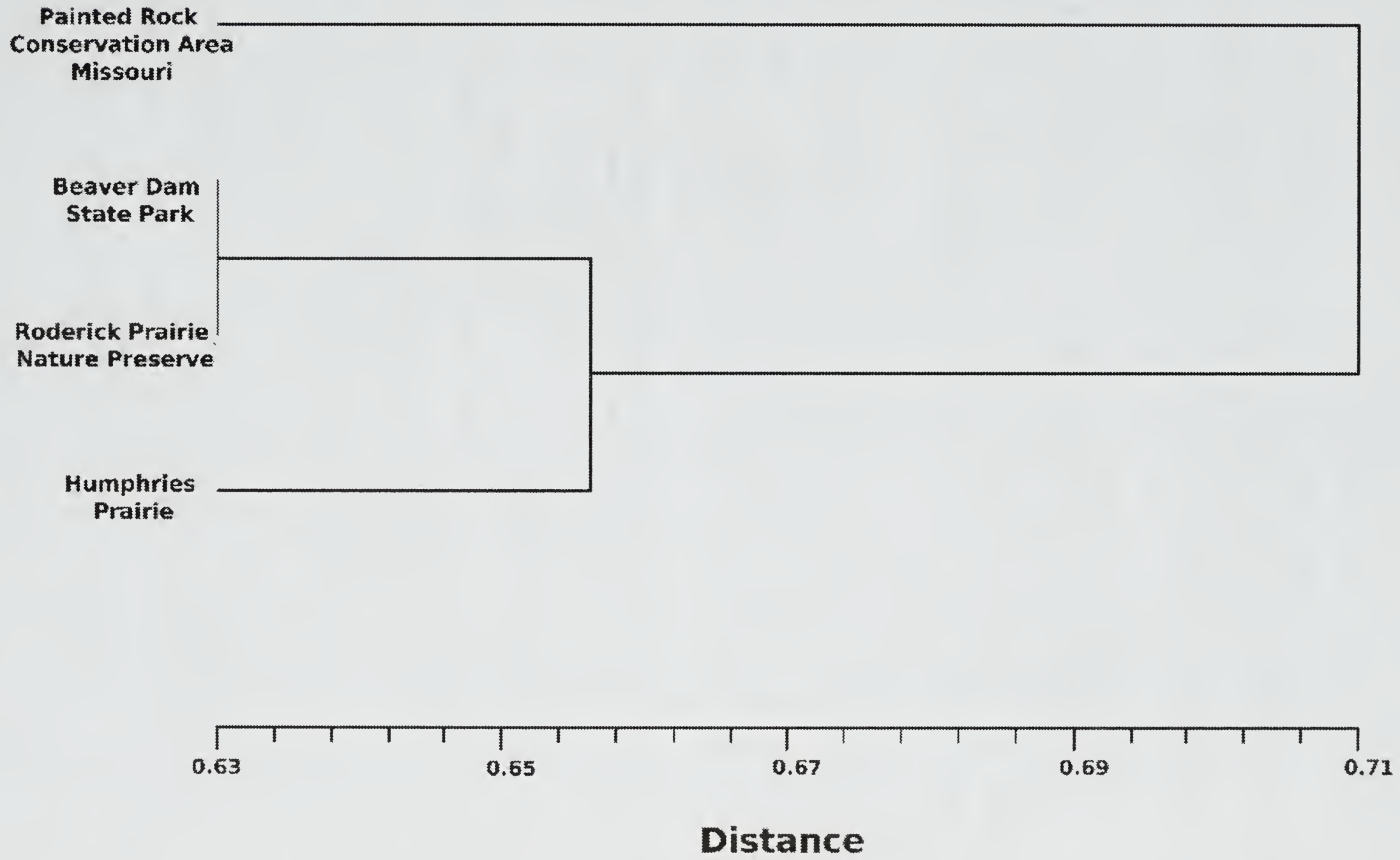
Figure 2. Phenogram of *Astragalus crassicarpus* var. *trichocalyx* based on ISSR distance values.



LITERATURE CITED

Alexander, J. A., A. Liston and S. J. Popovich. 2004. Genetic diversity of the narrow endemic *Astragalus oniciformis* (Fabaceae). *American Journal of Botany*. **91**:2004-2012.

Anderson, Sydney. 1994. Area and endemism. *The Quarterly Review of Biology*. **69**(4):451-471.

Barneby, R.C. 1964. Atlas of North American *Astragalus*. *Memoirs of the New York Botanical Garden*.

Baskin, C. C., and E. Quarterman. 1969. Germination requirements of seeds of *Astragalus tennesseensis*. *Bulletin of the Torrey Botanical Club,* **96:** 315-321.

Crawford, D.J., E. J. Esselman, J.L. Windus, and C.S. Pabin. 1998.Genetic variation in running buffalo clover (*Trifolium stoloniferum*: Fabaceae) using random amplified polymorphic DNA markers (RAPDs) . *Annals of the Missouri Botanical Garden*. **85** (1):81-89.

Ebinger, J.E. and W.E. McClain. 1991. Forest succession in the prairie peninsula of Illinois. *Illinois Natural History Survey Bulletin*. 34.

Esselman, E.J., L. Jiangiang, D.J. Crawford, J.L. Windus, and A.D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. *Molecular Ecology*. **8** (3):443–451.

Godt, M.J.W., B.R. Johnson, and J.L. Hamrick. 1996. Genetic diversity and population size in four rare southern Appalachian plant species. *Conservation Biology*. **10**(3):796-805.

Godwin, I.D., E.A.B. Aitken, and L.W. Smith 1997. Application of Inter Simple Sequence Repeat (ISSR) markers to plant genetics. *Electrophoresis*. **18**:1524-1528.

Harris SA. 1999 RAPDs in systematics: a useful methodology? In: Hollingsworth PM, Bateman RM, Gornall RJ ed(s). *Molecular systematics and plant evolution*. (Systematics Association special volume; 57.)). London: Taylor and Francis pp.211-228

Herkert, J.R., and J.E. Ebinger. 2002. Endangered and Threatened Species of Illinois: Status and Distribution. *Springfield, Illinois: Endangered Species Protection Board.*

Illinois Department of Natural Resources (IDNR). 2007. Illinois wildlife action plan. *Department of Natural Resources*. 5 October 2007. <http://www.dnr.state.il.us/orc/Wildliferesources/theplan/>

Illinois Department of Natural Resources. 2007. IDNR division of natural heritage home page. 20 November 2007. <http://dnr.state.ilus/conservation/naturalheritage/florafauna/document.htm>

Illinois Natural Heritage Database (INHD). 2007. Illinois threatened and endangered species by county. *Illinois Department of Natural Resources*. 20 November 2007. <http://dnr.state.il.us/conservation/naturalheritage/inhd.htm>

Karron, J. D., Y. B. Linhart, C. A. Chaulk and C. A. Robertson. 1988. Genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). American Journal of Botany **75**: 1114-1119.

Kearns, C.A., D.W. Inouye, and N.M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annu. Rev. Ecol. Syst*. **29**:83–112.

McClain, W.E. M.A. Phipps, H.H. Eilers and J.E. Ebinger. 2002. Vascular plants of glacial drift prairies in Macoupin County, Illinois. *Castanea*. **67**:54-60.

McClain, W.E. and J.E. Ebinger. 2003. Genus *Astragalus* (Fabacaeae) in Illinois. *Transactions of the Illinois State Academy of Science*. **97**(1):11-18.

McGlaughlin, M, K. Karoly, and T. Kaye. 2002. Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, *Abronia umbellata* subsp. *breviflora* (Nyctaginaceae). Conservation Genetics. **3**: 411-420.

McGregor, R.L. 1986. Flora of the Great Plains (T.M. Barkley, Ralph E. Brooks, and Eileen K. Schofield, Eds.). *Lawrence: University Press of Kansas*.

Nagaoka, T. and Y. Ogihara. 1997. Applicability of Inter-Simple Sequence Repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and Applied Genetics*. **94**:597-602.

Popovich, S. and D. A. Pyke. 1997. Impacts of wildfire rehabilitation and plow-and-seed land treatments on fitness parameters of an endemic milkvetch. Pages 27-36, In: Greenlee, J.M. (ed.), Proceedings -- fire effects on rare and endangered species and habitats conference, Nov. 13-16, 1995. International Association of Wildland Fire, Fairfield, WA.

Reveal, J.L. 1978. Astragalus phoenix Barneby (Ash Meadows milk-vetch). Status Report. *U. S. Fish and Wildl. Serv., Portland, OR.*

Rogenski, C., G. Wells, and M. Smith. 2004. Soil seed bank for *Astragalus crassicarpus* var. *trichocalyx* in Macoupin County. *Transactions of the Illinois State Academy of Science*. **97**:46.

United States Department of Agriculture (USDA). 2007. Missouri county level distribution for *Astragalus crassicarpus* var. *trichocalyx* (groundplum milkvetch). *United States Department of Agriculture: Natural Resources Conservation Service*. 20 November 2007. <http://plants.usda.gov/java/countystate_name=Missouri&statefips=29&symbol=ASCRT>

USDA. 2007. Plants profile for *Astragalus crassicarpus* var. *trichocalyx* (groundplum milkvetch). *United States Department of Agriculture: Natural Resources Conservation Service*. 20 November 2007. <http://plants.usda.gov/java/profile?symbol=ASCRT>

USDA. 2007. Plants profile for *Astragalus crassicarpus* (groundplum milkvetch). *United States Department of Agriculture: Natural Resources Conservation Service*. 20 November 2007. <http://plants.usda.gov/java/profile?symbol=ASCR2>

U.S. Fish and Wildlife Services (USFWS). 2007. USFWS: Endangered plants in the upper midwest (region 3). 12 December 2007. <http://www.fws.gov/midwest/endangered/plants/>

Wells, G.G. 2006. Life history and reproductive biology of *Astragalus crassicarpus* var. *trichocalyx*. M.S. Thesis. *Southern Illinois University Edwardsville*.

Wirth, T.A. & D.A. Pyke. 2003. Restoring forbs for sage grouse habitat: fire, microsite and establishment methods. *Restoration Ecology* **11**:370-377.

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Subcellular Centrin Localization within Distinct Compartments of *Vorticella convallaria*

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ABSTRACT

The stalked ciliated protozoan, *Vorticella convallaria*, uses a mode of contraction that differs from most other eukaryotic motile systems. Because calcium alone induces this contraction, we have undertaken the molecular characterization of the calcium-binding proteins, centrins/spasmins, associated with the contractile organelles in this organism. Many species have evolved centrin/spasmin multi-gene families with the prediction of an increased range of functions among these organisms. To define these functions in *V. convallaria*, we have initiated immunolocalization studies at the light and the ultrastructural level utilizing anti-centrin and anti-stalk antibodies. We have localized these centrin/spasmin antibodies to various contractile cytoskeletal structures within the cell and our transmission electron microscopy localization data indicate a further restriction to compartments within these organelles. The results obtained from this study allowed us to present a model in which we hypothesize the mechanism of contraction and relaxation in this ciliated protozoan and allows us to begin to dissect the function of this multi-gene family.

Key Words: Calcium-binding proteins, calcium-induced contraction, contractile organelles, fibrillar mass, membrane-bounded tubules, myoneme, nanofibers, spasmoneme

INTRODUCTION

Vorticella convallaria is a polymorphic, ciliated protozoan that spends the majority of its life cycle as an actively-feeding, sessile cell attached to a substrate by its contractile stalk (Fig. 1A). In nature, a mechanical stimulation of these cells results in a rapid contraction of the cell body and stalk (Fig. 1B). This contraction is brought about by an integrated, cytoskeletal fiber system (myonemes and spasmoneme) that transverses from the oral opening, through the cell body and terminates at the end of the stalk (Amos 1972). The myonemes are the fiber system within the cell body that are bundled together to form the spasmoneme, the stalk's contractile organelle (Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). Ultra-structural studies showed the myonemes and the spasmoneme

are morphologically identical (Allen 1973; Amos 1972; Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). Additionally, these studies revealed two distinguishable regions within these organelles: a "fibrillar mass" composed of longitudinally oriented 2-5 nm fibers and "membrane-bounded tubules" evenly distributed throughout the fibrillar mass (Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). Interestingly, the membrane-bounded tubules are also filled with longitudinally oriented 2-5 nm fibers (Allen 1973; Amos 1972). The biochemical composition of these fibers and the role they play in the fibrillar mass and within the membrane-bounded tubules are still unknown. Our research is focused on understanding the molecular basis of these fibers and their possible function in this unique contractile system.

During contraction, the stalk coils to 20-30% of its extended length in 2-4 milliseconds and biochemical studies have demonstrated that this contraction is calcium-driven and ATP-independent (Amos 1971; Amos 1972; Hoffman-Berling 1958). Amos showed that a change from 10^{-8} M to 10^{-6} M calcium was sufficient to induce contraction of isolated stalks (Amos 1971). Supporting the importance of calcium in contraction, Carasso and Favard (1966a; 1966b) localized calcium within the membrane-bounded tubules and postulated a calcium storage/release role for this compartment. Biochemically, Amos et al. (1975) determined that the major constituents of the spasmoneme were small, acidic calcium-binding proteins, denoted spasmins. He proposed they formed the 2-5 nm fibers found within this organelle. Maciejewski et al. (1999) isolated a *Vorticella* spasmin cDNA and showed its evolutionary relatedness to another family of small, acidic EF-hand calcium-binding proteins, centrins.

It is now well-established that centrin proteins are present in all eukaryotic cells and involved in a number of processes in the cell, but its molecular mechanism of action is still poorly understood. Centrins are localized to the centrioles/basal bodies where they function in the duplication (Baum et al. 1986; Koblenz et al. 2003; Salisbury et al. 2002; Stemm-Wolf et al. 2005) or the proper positional orientation of this organelle (Ruiz et al. 2005). Additionally, centrins have been associated with a number of other cellular processes: DNA repair via nucleotide excision repair (Araki et al. 2001; Molinier et al. 2004), nuclear mRNA export (Fischer et al. 2004), and signal transduction (Pulvermuller et al. 2002). Finally, centrins are integral components of calcium-triggered contractile fibers (Hart and Salisbury 2000; Levy et al. 1996). In many protists, these fibers systems have been expanded into elaborate contractile cytoskeletons with a concomitant increase in the number of centrin genes. For instance, in *Paramecium*, the contractile cytoskeleton (the infraciliary lattice) is mainly composed of nine centrin subfamilies and two subfamilies of a high molecular weight centrin-binding Sfi1p-like protein (Gogendeau et al. 2007; Gogendeau et al. 2008). The recent identification of Sfi1p and Sfi1p-like proteins suggests that the centrin-associated contractile fibers are composed of two units (Kilmartin 2003; Salisbury 2004). This model proposes a Sfi1p/centrin fiber with Sfi1p constituting the flexible backbone and centrins inducing the calcium-regulated contraction. The conformational change in the centrin protein, upon binding calcium, exerts torsional forces along the Sfi1p-backbone and results in a local twisting, bending, and shortening of the fiber. Recent support for this model has been demonstrated in the contractile infraciliary lattice of *Paramecium* where Sfi1-like proteins and various centrins mediate a calcium-responsive contraction of the cytoskeletal network (Gogendeau et al. 2007). In *Vorticella*, a similar expansion of a centrin gene family has been identified and consists of at least

one spasmin and six centrins (Maciejewski et al. 1999; McCutcheon et al. 2002). However, the exact nature of the 2-5 nm fibers, the function of the multiple centrins/spasmins or the presence of a *V. convallaria*-like Sfi1p are unknown. Additionally, the morphology of *Vorticella*'s cytoskeleton is more complex with membrane-bounded tubules embedded within the fibrillar mass. It has been proposed that the membrane-bounded tubules are calcium storage/release compartments but no explanation was given for the presence of 2-5 nm fibers within the tubules. The various fibers systems within *Vorticella* make it a good organism to address the various roles of centrins/spasmins within the contractile fiber and may yield insights into their mode of action. Therefore, we initiated an ultrastructural study of two *V. convallaria* centrin/spasmin proteins to begin to address these questions. Our results will show that both *V. convallaria* centrin/spasmin localize to the contractile cytoskeleton yet have a differential distribution within these organelles. A novel model incorporating the complex morphology with the current view of centrin/spasmin-based contraction is discussed.

MATERIALS AND METHODS

Cell culture

Vorticella convallaria cells were grown in a bacteria-containing medium of boiled wheat grass according to the methods of Vacchiano et al. (1991). We obtained large number of cells ("rejuvenated cells") by replacing the culture medium with fresh medium and allowing cell growth for 48 hrs, replacing the medium again and shaking the culture for 24 hrs. All cells used in our experiments were "rejuvenated cells."

Antibodies

1F5-antibody is a mouse monoclonal antibody produced against isolated *V. convallaria* stalk protein (Vacchiano 1992). N-term*Vc*Centrin4-antibody, a polyclonal rabbit antibody, was raised against a synthetic peptide of the derived N-terminal sequence of *Vc*Centrin4 (McCutcheon et al. 2002). FITC-conjugated goat anti-rabbit and anti-mouse antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Gold conjugated goat anti-rabbit (5 nm) and anti-mouse (15 nm) antibodies were purchased from Ted Pella, Inc. (Redding, CA). Alkaline Phosphatase (AP)-conjugated anti-rabbit antibody was purchased from Promega Co. (Madison, WI) and AP-conjugated anti-mouse antibody was purchased from Jackson ImmunoResearch Laboratories. 1F5-antibody, a mouse monoclonal antibody detects a recombinantly expressed spasmin protein (Vacchiano 1992).

Electrophoresis and western blot

V. convallaria protein preparations from whole cell lysates were isolated using Tri Reagent (Molecular Research Center, Inc. Cincinnati, OH) according to manufacturer's instructions. BioRad Tris-HCl Criterion Precast Gels (18%) were used for SDS-gel electrophoresis and for western analysis. The gels were electrophoresed at a constant voltage of 200 V for 1hr 15 min. Following electrophoresis, the separated proteins were transferred to Immuno-blot PVDF membrane (Bio-Rad Laboratories, Hercules, CA) using the BioRad Criterion Blotter and processed for western analysis using BioRad Immun-Star^{TM}AP Substrate according to manufacturer's instructions. The primary antibody dilutions were as follows; 1:2,000 for N-term*Vc*Centrin4-antibody and 1:100 for 1F5-anti-

body and 1:6,500 for anti-rabbit IgG-AP conjugate and 1:5,000 for anti-mouse IgG-AP conjugate.

Immunofluorescent studies

For immunofluorescent studies, rejuvenated cells (see above) were allowed to attach overnight to coverslips. Prior to fixation, the cells were exposed to relaxation buffer (10 mM EGTA, 50 mM Tris-base, 10 mM KPO_4, 7.5 mM NH_4Cl, 240 μM KCl, 240 μM $MgSO_4$, 0.2% Triton X-100) for two min. They were then fixed in 100% methanol containing 100 μl of 24 mM $MgSO_4$ and 100 μl of 24 mM KCl per 10 ml at -20°C for 10 min. Following three 5-min washes in modified phosphate buffered saline (PBS) buffer (130 mM NaCl, 2 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 10 mM EGTA, 2 mM $MgCl_2$, 0.1% Tween 20 at pH 7.4), the attached cells were incubated in the primary antibody (1:1,000 for N-term*Vc*Centrin4-antibody and 1:10 for 1F5-antibody) for one hr. Cells were then washed three times in modified PBS buffer for 10 min and incubated in secondary antibody (1:500 dilution for both antibodies) for 30 min. Following the three 10-min washes in PBS, the cells were mounted in Vectashield mounting medium (Vector Laboratories Inc., Burlingame, CA). The treated coverslips were placed on glass slides and viewed using a ZeissAxioskop microscope equipped with phase and fluorescent objectives.

Transmission Electron Microscopy

V. convallaria cells were fixed in 2% paraformaldehyde, 0.1% glutaraldehyde, and 0.1 M cacodylate (pH 7.2), then transferred through a series of acetone dehydrations (30, 50, 75, 90, and two washes in 100% acetone) under vacuum in a PELCO BioWave™ 34700 microwave oven (Ted Pella, Inc., Redding, CA). Each cycle consisted of 40 sec at 100% power followed by one min at 0% power. LR White Resin (London Resin Company Ltd., Reading, Berkshire RG7 4YG England) infiltration consisted of three changes (10 min at 100% power, under vacuum) of acetone to resin: 2:1, 1:1, 1:2. The resin was polymerized at 80°C for 45 min without vacuum. Eighty nm sections were produced using a Reichert Ultracut E microtome (Leica, Deerfield, IL) and collected on 200-mesh nickel grids (Ted Pella, INC., Redding, CA) for immuno-gold processing. The grids were blocked for 45 min in blocking buffer (0.8% BSA, 0.1% IGSS quality gelatin, 5% non-immune serum in PBS, pH 7.4) and washed twice for five min each using wash buffer (0.8% BSA, 0.1% IGSS quality gelatin in PBS, pH 7.4). The grids were then incubated with primary antibodies overnight [N-term*Vc*Centrin4 (1:50, 1:100 dilution of 5 nm gold conjugated anti-rabbit) and 1F5 (1:2.5, 1:75 dilution of 15 nm gold conjugated anti-mouse)]. Four 5-min washes in wash buffer were applied followed by incubation with gold-conjugated secondary antibodies for four hrs. Again, the grids were taken through six-5 min washes in wash buffer and two-5 min washes in PBS (pH 7.4). The grids were then exposed to 2% glutaraldehyde in PBS (pH 7.4) for ten minutes and washed two times in double deionized water for five minutes. The grids were post-stained in 2% aqueous uranyl acetate for five min, followed by one min in lead citrate and viewed on a JOEL 1200E electron micrscope. The distribution of labeled antibodies was analyzed by examining printed micrographs.

In order to determine the respective areas occupied by the fibrillar mass and the tubules, *Vorticella* cells were fixed under conditions that are optimal for staining membranes using a protocol by David H. Hall. The protocol is found at the following website:

http://neuroscience.aecom.yu.edu/labs/halllab/wormem/methods.htm. Following fixation, eighty nm sections were produced and viewed on a JOEL 1200E electron microscope. Ten micrographs (8 X 11 in) were enlarged 25 times and the percent ratio of membrane-bounded tubules to fibrillar mass within the spasmoneme was determined using an area meter model LI-3100 (LI-COR, Inc., Lincoln, NE). The total surface area of the spasmoneme was measured then the area of the membrane-bounded tubules and fibrillar mass was determined.

RESULTS AND DISCUSSION

Previously, we identified a multi-gene family of centrin/spasmin (Maciejewski et al. 1999; McCutcheon et al. 2002; Vacchiano 1992) proteins in *V. convallaria*. Because our primary interest is concerned with dissecting the role of centrins/spasmins in cytoskeletal contraction, we focused initially on localization (by immunofluorescence) and distribution (by immuno EM) of two centrin/spasmin proteins using two antibodies, N-term*Vc*Centrin4-antibody and 1F5-antibody. The 1F5-antibody is one of several monoclonal antibodies isolated from a mouse monoclonal antibody screen using enriched stalk proteins as the antigen (Vacchiano, 1992). The 1F5-antibody was characterized and detected a *V. convallaria* calcium-binding protein (Vacchiano, 1992). Subsequently, this antibody was shown to recognize a recombinantly expressed *V. convallaria* spasmin protein (Maciejewski et al., 1999). Western blot analysis, using protein from *Vorticella* whole cell lysates, was performed to confirm that each antibody recognized distinct *Vorticella* centrin/spasmin proteins. The western blot analysis (Fig. 2) indicates that N-term*Vc*Centrin4-antibody detects a single protein with a relative molecular weight of 22.5 kDa. 1F5-antibody recognizes two proteins with relative molecular weights of 23 and 19.6 kDa. Since the 1F5-antibody recognizes two proteins, the epitope is likely to a conserved calcium-binding domain. This data combined with previous results indicates that these antibodies recognize different *V. convallaria* centrin/spasmin proteins.

Since each antibody recognizes a distinct protein or a unique subset of proteins, these antibodies were used in cellular localization studies. At the light microscopy level, two contractile organelles, the myonemes and spasmoneme, are detected by both antibodies (Fig. 3). The myonemal fibers transverse the cell body and as they approach the posterior end (scopular region) of the cell body, they bundle together to form one massive spasmoneme that traverses through the length of the stalk. Although these contractile fibers are localized to different parts of the cell, cell body versus stalk, they are morphologically identical and perform the same function which is rapid contraction of the whole organism (Allen 1973; Amos 1972; Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). The immunofluorescent studies revealed that the myonemal fibers of the cell bodies are strongly labeled by both N-term*Vc*Centrin4-antibody and 1F5-antibody (Fig. 3). Using the descriptive myonemal fiber system developed for another peritrich ciliate *Campanella umbellaria* (Shi et al. 2004), our study shows N-term*Vc*Centrin4-antibody labels the *Vorticella* myonemal system composed of longitudinal fibers running from the base of the peristomial disc/lip of the cell body to the junction between the scopular lip and the stalk (Fig. 3). The labeling pattern reveals that the longitudinal myonemes are uniformly distributed around the cell body with a separation of approximately 1.4 μm, and they bifurcate along their length forming interconnecting branches linking individual longitudinal myonemes to one another resulting in a web-like fiber network. The longitudinal myo-

nemes are, in turn, connected with the peristomial myonemes that circumscribe the anterior part of the cell. In the contracted state, when the oral cilia are retracted inside the cell, the circular myonemes are most prominent. When the cell is relaxed, very delicate anastomosing fibers linking the peristomial myonemes are observed. A weak fluorescent signal is detected at the aboral end of the cell body localizing to the ciliary wreath. Additionally, the N-term*Vc*Centrin4-antibody localizes to the spasmoneme but the labeling is only revealed in the parts of the cell where the sheath was dislodged from the cell body. (At this point, we do not know why the labeling of the spasmoneme is only seen when the sheath is removed.)

The overall labeling pattern for the 1F5-antibody is similar to the N-term*Vc*Centrin4-antibody, albeit there are minute differences. First, though both antibodies label the whole length of the spasmoneme, the 1F5-antibody labels the spasmoneme without removing the extracellular sheath as was necessary to localize N-term*Vc*Centrin4-antibody to the spasmoneme. Second, the peristomial ring formed by the myonemes does not fluoresce as strongly as with the N-term*Vc*Centrin4-antibody. With the exception of these two minor variations, we were unable to discern any other differences between the localization of these antibodies at the light microscopic level. At this resolution level, the colocalization of these proteins to the same subcellular compartment was unclear, so we initiated ultrastructural studies to further define the localization of these two antibodies.

The most interesting finding is that our data show a differential localization of these proteins to distinct sub-compartments within the spasmoneme (Fig. 4-7). 1F5-antibody localizes almost exclusively to the fibrillar mass of the spasmoneme, while the N-term*Vc*Centrin4-antibody labels both the fibrillar mass and the membrane-bounded tubules. Quantification of the gold particle distribution showed that 26% of N-term*Vc*Centrin4-antibody grains are located inside the membrane-bounded tubules and 68.5% localize within the fibrillar mass (Fig. 8). For the 1F5-antibody, 93.5% of the gold particles localized to the fibrillar mass and only 6.5% were distributed inside the membrane-bounded tubules (Fig. 8). Localization of centrin/spasmin proteins within the fibrillar mass, the major contractile force of the spasmoneme, is consistent with their association with contractile fibers. However, (even though the membrane-bounded tubules contain a fibrous core) the localization of a centrin protein within the membrane-bounded tubules would not be predicted from previous models of the tubules as simple calcium storage/release compartments (Carasso and Favard 1966a; 1966b). Based on this observation, we hypothesize that besides a calcium storage/release role, the membrane-bounded tubules have a structural function within the spasmoneme and a centrin/spasmin protein plays an important role in this process.

If the membrane-bounded tubules have a structural role, then they should constitute a significant cross-sectional area of the spasmoneme. Results from our morphological investigation, a finding consistent with previous studies, revealed the spasmoneme is composed of a fibrillar mass and membrane-bounded tubules containing 2-5 nm fibers (Fig.7) (Allen 1973; Amos 1972). Our analysis of these subcomponents showed that the membrane-bounded tubules represent approximately 15% and the fibrillar mass 85% of the area of the spasmoneme. Additionally, the membrane-bounded tubules appear continuous through the whole length of the spasmoneme. The even dispersal of the membrane-bounded tubules, the significant cross-sectional area occupied by them, and the

longitudinal extension of these structures throughout the spasmoneme suggest a potential structural function for these substructures in addition to the proposed calcium storage/release function.

We propose a novel, testable model that incorporates the calcium storage/release function of the membrane-bounded tubules and explains the presence of fibers within the fibrillar mass and in the membrane-bounded tubules and the distribution of the centrin/spasmin antibodies. In our model, the membrane-bounded tubules serve two roles, as calcium storage/release sites and as structural support for the spasmoneme. This duel function facilitates both the contraction (calcium release, fiber disassembly) and extension (calcium uptake, fiber reassembly) of the spasmoneme. We propose that the tubular fibrils are formed by self-assembled centrin/spasmin proteins (Wiech et al. 1996; Yang 2006; Zhao et al. 2009) while the fibrillar mass fibers are composed of bundles of Sfi1-like proteins with bound centrin/spasmin proteins analogous to the contractile infraciliary lattice in *Paramecium* (Gogendeau et al. 2007). During stalk contraction, calcium released from the membrane-bounded tubules into the fibrillar mass initiates contraction through cumulative conformational changes of the fibrillar fibers caused by calcium binding to centrin/spasmin which in turn initiates torsional forces along the Sfi1p-backbone resulting in their twisting and shortening as first proposed by Salisbury (Salisbury 2004). Within the membrane-bounded tubules following calcium release, the aggregated centrin/spasmin fibers disassemble and the membrane-bounded tubules lose their rigidity offering minimal resistance during spasmonemal contraction. During stalk relaxation, the calcium concentration within the tubules increases, initiating self-aggregation of centrin/spasmin proteins into a meshwork of fibers that lends structural integrity and assists in the lengthening of the stalk by stiffening of the spasmoneme. The self-assembly of centrins (Yang 2006; Zhao et al. 2009) and aggregation in elevated calcium has been reported (Wiech et al. 1996; Yang 2006). Therefore, the centrin/spasmin proteins with their calcium-binding properties would participate in both the contraction and the relaxation cycles of this unique contractile system. To test our hypothesis, we plan to identify and characterize a *Vorticella*'s Sfi1 protein(s). Our model predicts the Sfi1p will localize exclusively to the fibrillar mass.

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LITERATURE CITED

Allen, R.D. 1973. Contractility and its control in peritrich ciliates. *J of Protozool* 20: 25-36.

Amos, W.B. 1971. Reversible mechanochemical cycle in contraction of *Vorticella*. *Nature*, 229: 127-128.

Amos, W.B. 1972. Structure and coiling of the stalk in the peritrich ciliates *Vorticella* and *Carchesium*. *J of Cell Sci*, 10: 95-122.

Amos, W.B., Routledge, L.M., and Yew, F.F. 1975. Calcium-binding proteins in a vorticellid contractile organelle. *J Cell Sci*, 19: 203-212.

Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., Ohkuma, Y., and Hanaoka, F. 2001. Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *Journal of Biological Chemistry*, 276(22): 18665-18672.

Baum, P., Furlong, C., and Byers, B. 1986. Yeast gene required for spindle pole body duplication: homology of its product with Ca2+-binding proteins. *Proc Natl Acad Sci USA*, 83: 5512-5516.

Carasso, N. and Favard, P. 1966a. *Le reticulum endoplasmiques dans les myonemes de ciliés peritrices et son role dans l'accumulation du calcium*. Maruzen Company, Ltd., Tokyo.

Carasso, N. and Favard, P. 1966b. Mise en évidence du calcium dans les myonemes pédoncularies de ciliés péritriches. *J of Micros*, 5: 759-770.

Fauré-Fremiet, E., Rouiller, C., and Gauchery, M. 1956. Les Structures Myoïdes Chez Les Ciliés. Étude au Microscope Électronique. *Arch Anat Micros*, 45: 139-161.

Fischer, T., Rodriguez-Navarro, S., Pereira, G., Racz, A., Schiebel, E., and Hurt, E. 2004. Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. *Nature Cell Biology*, 6(9): 840-U844.

Gogendeau, D., Beisson, J., de Loubresse, N.G., Le Caer, J.-P., Ruiz, F., Cohen, J., Sperling, L., Koll, F., and Klotz, C. 2007. An Sfi1p-Like Centrin-Binding Protein Mediates Centrin-Based Ca2+-Dependent Contractility in *Paramecium tetraurelia*. *Eukaryot Cell* 6(11): 1992-2000.

Gogendeau, D., Klotz, C., Arnaiz, O., Malinowska, A., Dadlez, M., Garreau de Loubresse, N., Ruiz, F., Koll, F., and Beisson, J. 2008. Functional diversification of centrins and cell morphological complexity. *Jounal of Cell Science*, 121(1): 65-74.

Hart, P.E. and Salisbury, J.L. 2000. Molecular aspects of the centrin-based cytoskeleton in flagellates. in *Flagellates: Unity, Diversity and Evolution* (ed. B.S.C. Leadbeater and J.C. Green), pp. 95-109. Taylor and Francis, London, New York.

Hoffman-Berling, H. 1958. Der muchanismus einew neuen, von der muskelkontraktion vershiedenen, kontraktionzyklus. *Biochem and Biophys Acta*, 27: 247-255.

Kilmartin, J.V. 2003. Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. *J Cell Biol*, 162(7): 1211-1221.

Koblenz, B., Schoppmeier, J., Grunow, A., and Lechtreck, K.F. 2003. Centrin deficiency in *Chlamydomonas* causes defects in basal body replication, segregation and maturation. *Journal of Cell Science*, 116(13): 2635-2646.

Levy, Y.Y., Lai, E.Y., Remillard, S.P., Heintzelman, M.B., and Fulton, C. 1996. Centrin is a Conserved Protein that Forms Diverse Associations with Centrioles and MTOCs in *Naegleria* and Other Organisms [Review]. *Cell MotilCytoskel*, 33(4): 298-323.

Maciejewski, J.J., Vacchiano, E.J., McCutcheon, S.M., and Buhse, H.E. 1999. Cloning and expression of a cDNA encoding a *Vorticella convallaria* spasmin: an EF-hand calcium-binding protein. *J Eukaryot Microbiol*, 46(2): 165-173.

McCutcheon, S.M., McLaughlin, N.B., and Buhse, H.E. 2002. Isolation and Characterization of a Centrin Multigene FamilyAssociated with the Contractile Cytoskeleton of *Vorticella convallaria*. Abstract. *Mol Biol Cell* 13: 331a.

Molinier, J., Ramos, C., Fritsch, O., and Hohn, B. 2004. Centrin2 modulates homologous recombination and nucleotide excision repair in Arabidopsis. *Plant Cell*, 16(6): 1633-1643.

Pulvermuller, A., Giessl, A., Heck, M., Wottrich, R., Schmitt, A., Ernst, O.P., Choe, H.W., Hofmann, K.P., and Wolfrum, U. 2002. Calcium-dependent assembly of centrin-G-protein complex in photoreceptor cells. *Molecular and Cellular Biology*, 22(7): 2194-2203.

Ruiz, F., de Loubresse, N.G., Klotz, C., Beisson, J., and Koll, F. 2005. Centrin deficiency in *Paramecium* affects the geometry of basal-body duplication. *Current Biology*, 15(23): 2097-2106.

Salisbury, J.L. 2004. Centrosomes: Sfi1p and centrin unravel a structural riddle. *Curr Biol*, 14(1): R27-R29.

Salisbury, J.L., Suino, K.M., Busby, R., Springett, M., 2002. Centrin-2 is required for centriole duplication in mammalian cells. *Current biology : CB*, 12(15): 1287-1292.

Shi, X., Warren, A., Yu, Y., and Shen, Y. 2004. Infraciliature and myoneme system of *Campanella umbellaria* (Protozoa, Ciliphora, Peritrichida). *J Morphol*, 261: 43-51.

Sotelo, J.R. and Trujillo-Cenóz, O. 1959. The Fine Structure of an elementary contractile system. *J Biophys Biochem Cytol*, 6: 126-128.

Stemm-Wolf, A.J., Morgan, G., Giddings, T.H., White, E.A., Marchione, R., McDonald, H.B., and Winey, M. 2005. Basal body duplication and maintenance require one member of the *Tetrahymena thermophila* centrin gene family. *Molecular Biology of the Cell,* 16(8): 3606-3619.

Vacchiano, E., Kut, J.L., Wyatt, M.L., and Buhse, H.E. 1991. A Novel Method for Mass-Culturing *Vorticella*. *J Protozool,* 38: 608-613.

Vacchiano, E.J. 1992. The establishment of Vorticella as a model system for the study of calfilamins. In *Dissertation*, pp. 155. University of Illinois at Chicago, Chicago, IL. Available on microfilm from University of Michigan, Accession number AAG9310158.

Wiech, H., Geier, B.M., Paschke, T., Spang, A., Grein, K., Steinkotter, J., Melkonian, M., and Schiebel, E. 1996. Characterization of Green Alga, Yeast, and Human Centrins - Specific Subdomain Features Determine Functional Diversity. *Journal of Biological Chemistry,* 271(37): 22453-22461.

Yang, A. 2006. The N-terminal domain of human centrin 2 has a closed structure, binds calcium with a very low affinity, and plays a role in the protein self-assembly. *Biochemistry,* 45(3): 880-889.

Zhao, Y., Song, L., Liang, A., and Yang, B. 2009. Characterization of self-assembly of *Euplotes octocarinatus* centrin. *Journal of Photochemistry and Photobiology B: Biology*, 95: 26-32.

Figure 1. Phase contrast images of *Vorticella convallaria*. A. Phase contrast image of a non-contracted fully extended cell. B. Phase contrast image of a fully-contracted cell.

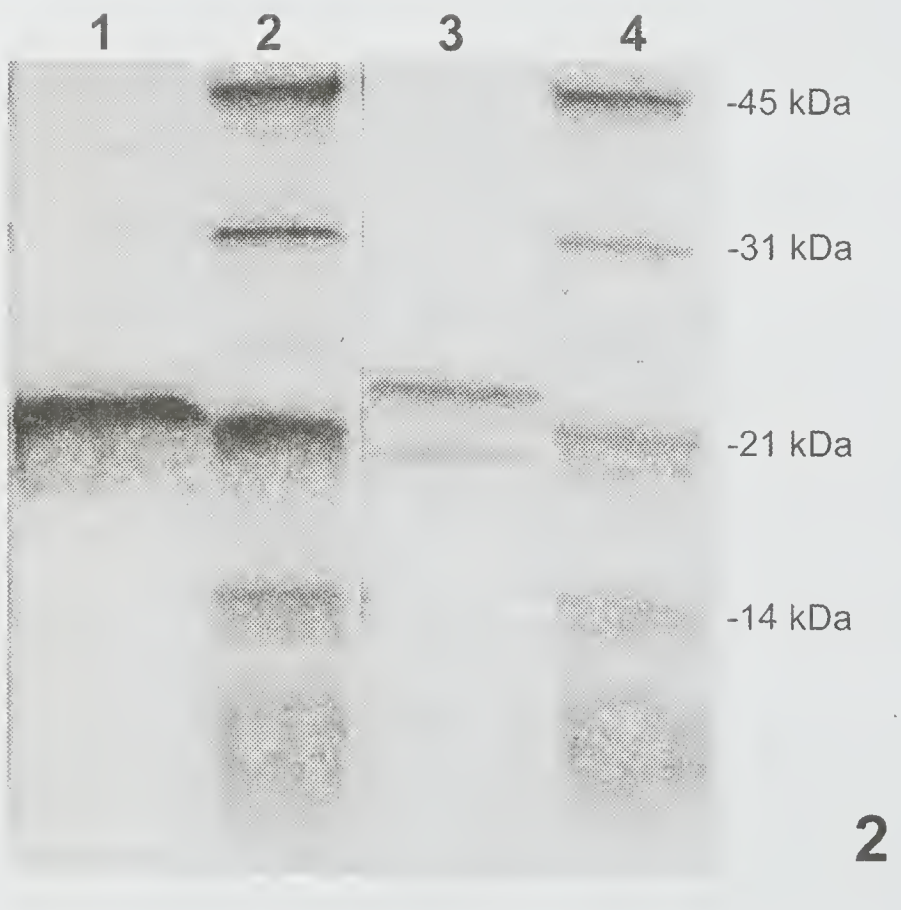

Figure 2. Immunodetection of centrin antibodies in *Vorticella convallaria*. Western blot analysis of N-term*Vc*Centrin4 and 1F5 antibodies. Lanes 1 and 3 contain *V. convallaria* whole cell lysates. Lanes 2 and 4 are standard markers whose molecular weights are indicated on the right. Lane 1 was probed with an anti-N-term*Vc*Centrin4-antibody and detected with Alkaline Phosphatase-conjugated anti-rabbit antibody. Lane 3 was probed with an anti-stalk 1F5-antibody and detected with Alkaline Phosphatase-conjugated anti-mouse antibody.

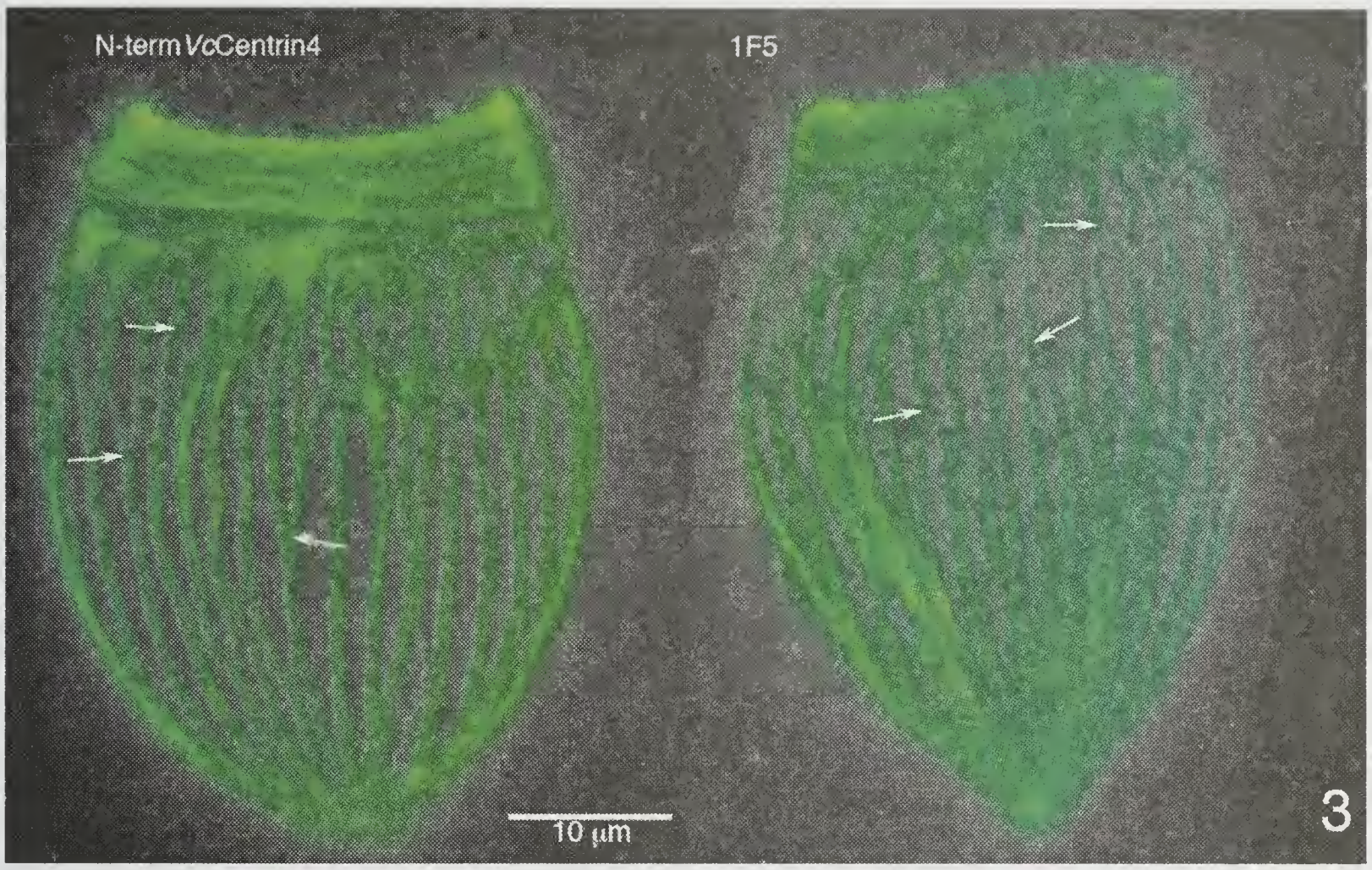

Figure 3. Contractile myonemes of *V. convallaria* shown at the light microscopy level. The cells were labeled with an anti-N-term*Vc*Centrin4-antibody or with an anti-stalk 1F5-antibody and detected with FITC-conjugated goat anti-rabbit antibody and FITC-conjugated goat anti-mouse antibody, respectively. For clarity, only the cell bodies are shown. The cells are oriented with the oral apparatus toward the top of the figure. Arrows indicate bifurcations along the longitudinal myonemes forming a web-like fiber network. Scale bar = 10 μm.

Figures 4-7. *Vorticella convallaria* spasmoneme ultrastructure.

Figures 4 & 5. TEM immuno-localization of N-term*Vc*Centrin4 (1:50, 1:100 dilution of 5 nm gold conjugated anti-rabbit) and 1F5 (1:2.5, 1:75 dilution of 15 nm gold conjugated anti-mouse) antibodies within the spasmoneme. FM denotes fibrillar mass. T designates membrane-bounded tubules. Individual tubules are indicated by arrows.

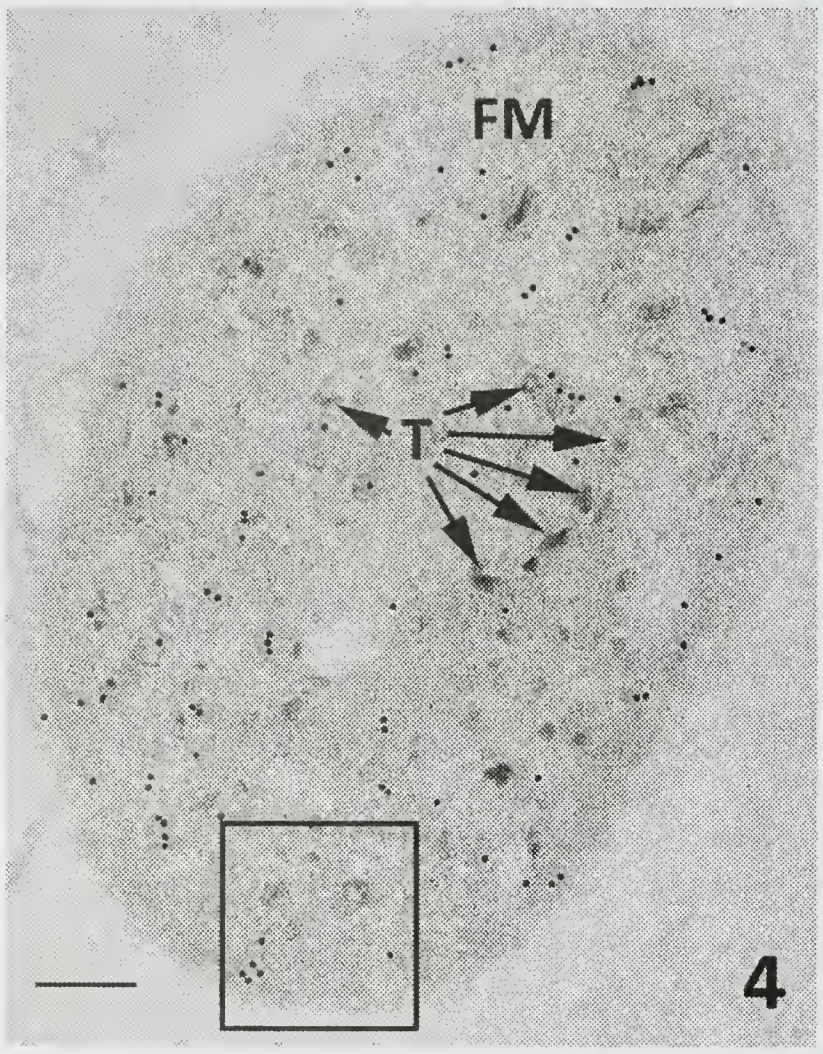

Fig. 4. Cross-section of the spasmoneme showing distribution of gold particles for double labeling with N-term*Vc*Centrin4 and 1F5 antibodies. At the ultrastructural level, the spasmoneme consists of two morphologically distinct compartments: the electron dense membrane-bounded tubules and the less dense fibrillar mass. Mag.= 20 K. Scale bar = 200 nm.

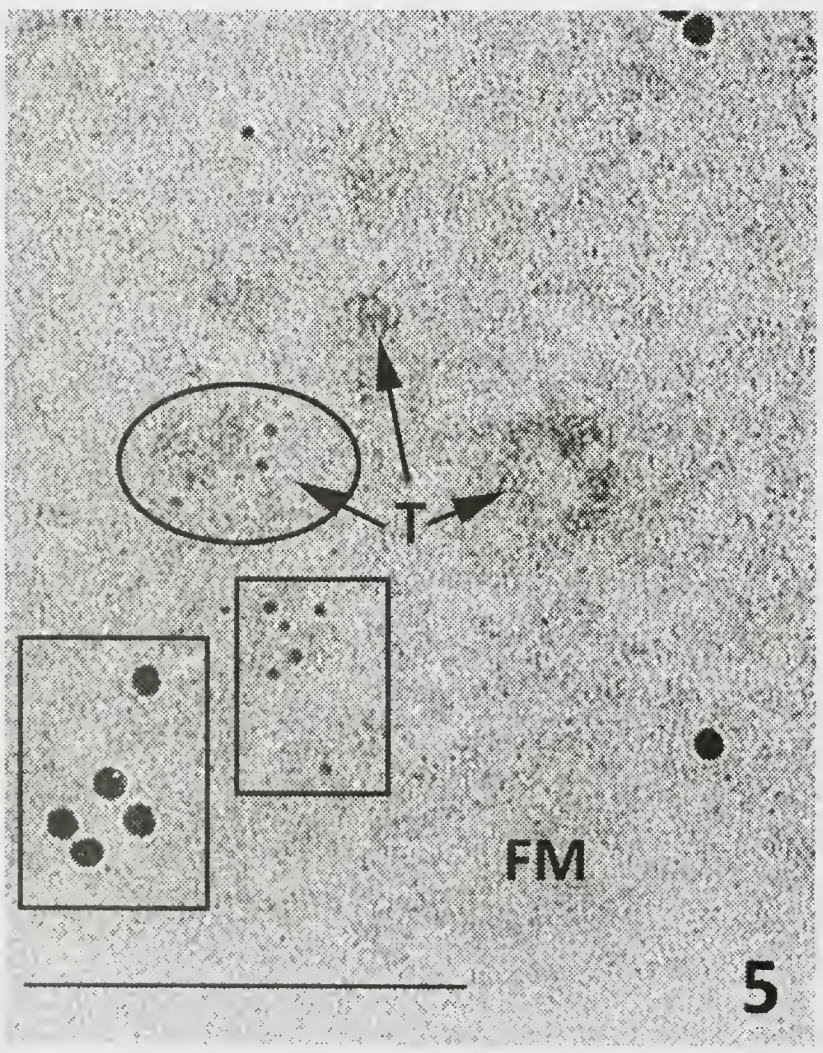

Fig 5. Higher magnification of the boxed area in Fig. 4. N-term*Vc*Centrin4 and 1F5 antibodies have distinct localizations within the spasmoneme. Their localization is indicated by ovals (within membrane-bounded tubules) and rectangles (within fibrillar mass). N-term*Vc*-Centrin4 antibody (5 nm gold) is found within the membrane-bounded tubules and the fibrillar mass. 1F5 antibody (15 nm gold) is localized almost exclusively to the fibrillar mass (see Fig. 8). Scale bar = 200 nm.

Figures 4-7, cont'd. *Vorticella convallaria* spasmoneme ultrastructure.

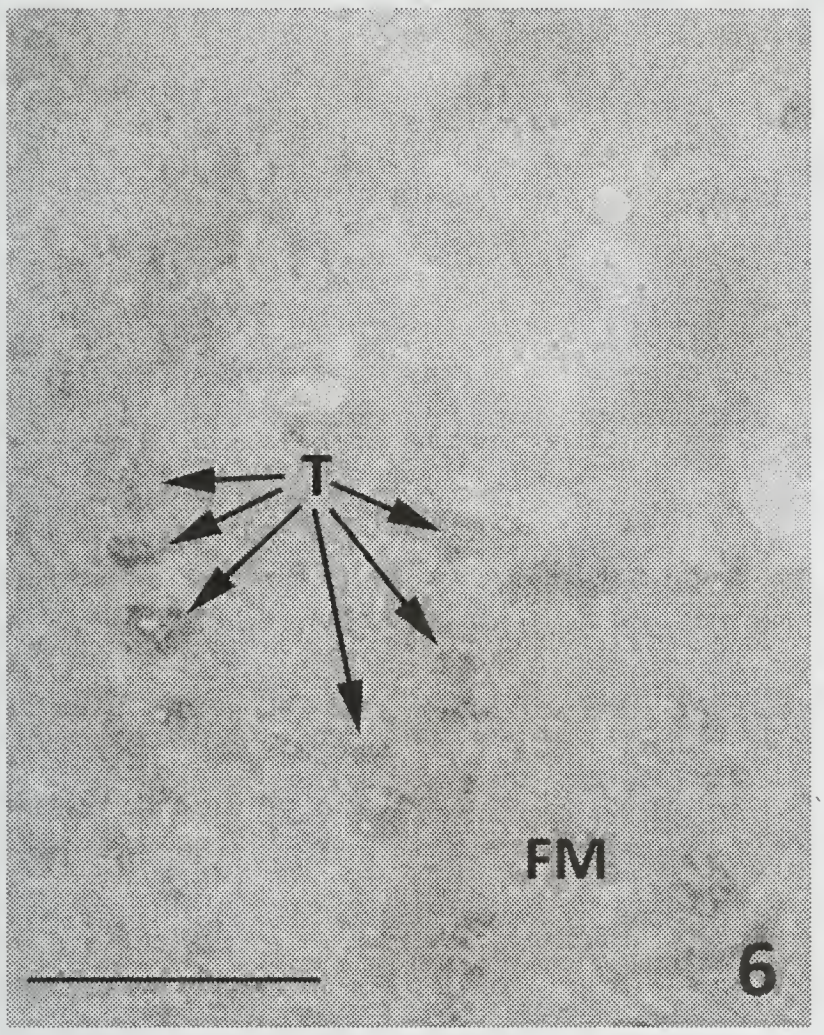

Fig 6. Cross-section of spasmoneme-negative control for double labeling with N-term*Vc*Centrin4 and 1F5 antibodies. Negative control grids were incubated with secondary antibodies only. Mag.= 30 K. Scale bar = 200 nm.

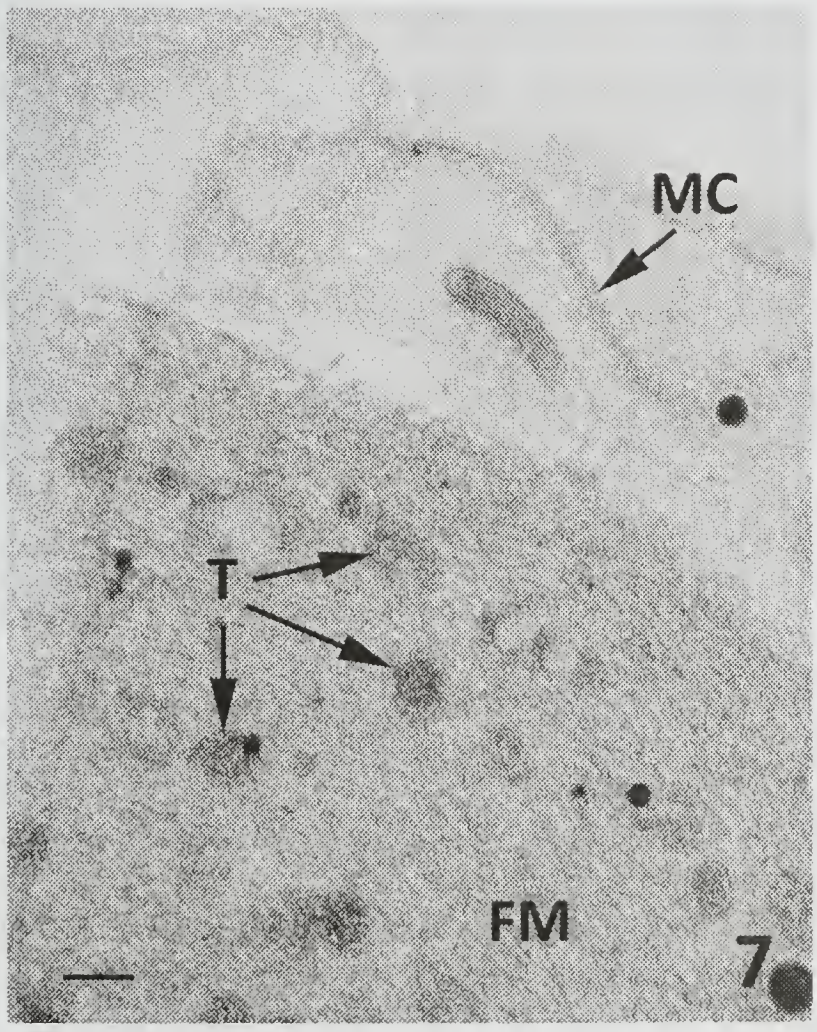

Fig 7. Ultrastructural morphology of the spasmoneme. Tangential TEM section showing a portion of the spasmoneme. The tubules are membrane-bounded. The membranes appear as halos around these electron dense structures which stand out from the background of fibrillar mass. FM denotes fibrillar mass. MC indicates the triple membrane complex (plasma membrane plus inner and outer membranes of alveolar sacs), a feature unique to all ciliates. T labels membrane-bounded tubules. Individual membrane-bounded tubules are indicated by arrows. Mag.=60 K. Scale bar =100 nm.

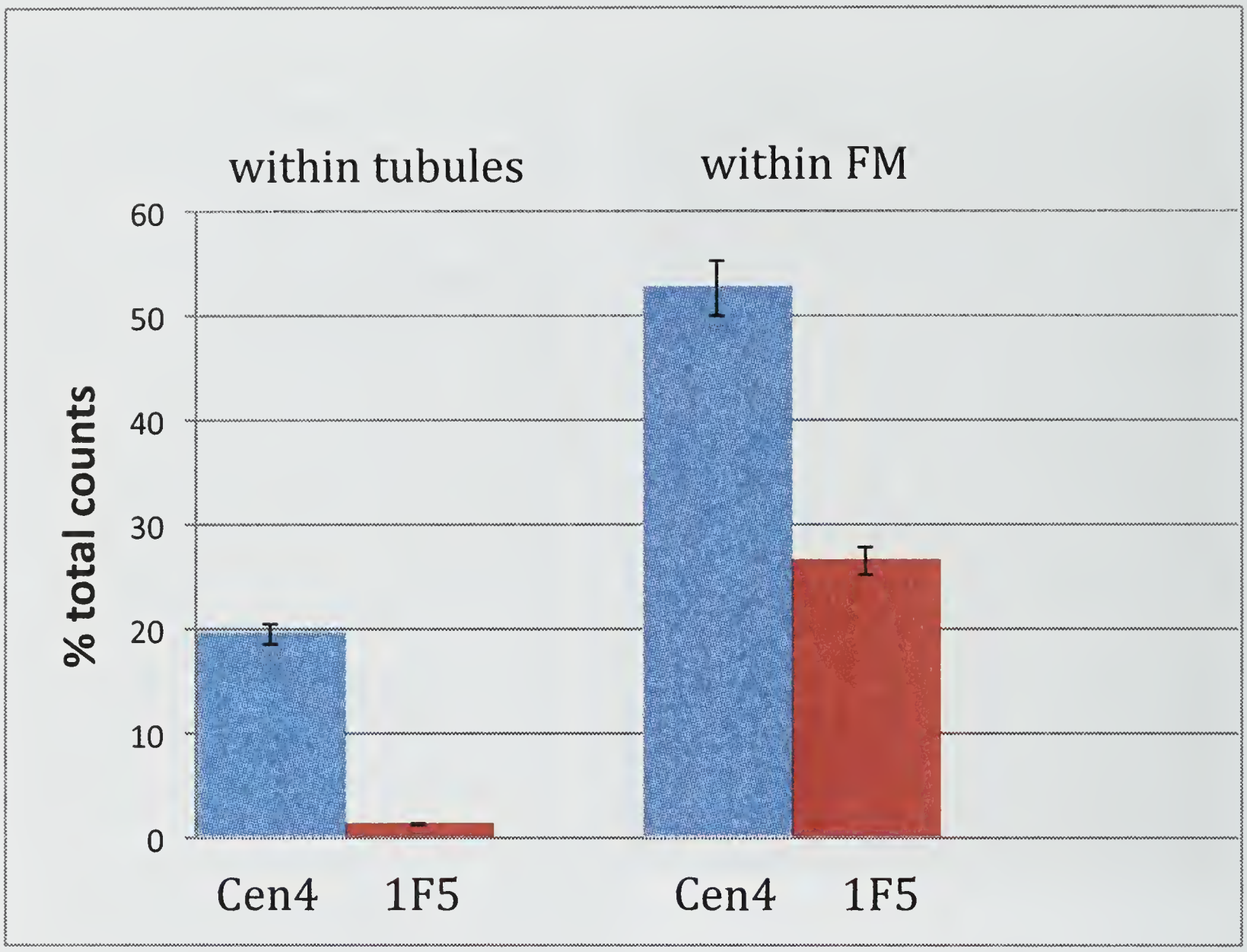

Figure 8. Analysis of the distribution of N-term*Vc*Centrin4 antibody and 1F5-antibody within the spasmoneme of *Vorticella convallaria*. The graph depicts results from five separate experiments that include analysis of thirty separate electron micrographs of the Vorticellan spasmoneme. Shown on the left is the distribution on N-term*Vc*Centrin4-antibody (blue) and 1F5-antibody (red) within the tubular compartment. On the right is the distribution of these antibodies within the fibrillar mass (FM) compartment. These data are presented as a percentage of the total number of gold particles that localize to the spasmomeme.

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Two Record Long Tornadoes in Illinois and Their Major Impacts

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ABSTRACT

The nation's two longest and most deadly tornadoes occurred in Illinois early in the 20th Century. One crossed central Illinois in 1917 and killed 211 persons while the other crossed southern Illinois in 1925 and killed 695, the nation's greatest loss of life from a tornado. These were the greatest natural disasters ever in Illinois. The 1917 tornado covered 293 miles and the 1925 storm covered 219 miles, both national records. Property damages in 1917 totaled $51.5 million (2008 dollars) and those in 1925 were $134.2 million (2008 dollars).

INTRODUCTION

The nation's two longest and most deadly tornadoes occurred in Illinois early in the 20th Century. In May 1917 a tornado began near Quincy and moved east across Illinois, killing 211 persons before moving on into Indiana. In March 1925 a tornado began in southeastern Missouri, moved across southern Illinois and entered southwestern Indiana, leaving 695 dead in Illinois. The loss of lives in 1925 still ranks as the highest ever by a tornado in the United States. These two storm events and their impacts are the worst natural disasters experienced in Illinois during the 20th Century. Both storms created long lasting impacts on society, science, and technology, and they are an interesting part of the state's history. The storms and their losses are described first, and this is followed by descriptions of the post-storm impacts and lessons learned in ensuing decades.

Illinois averages 26 tornadoes per year but is not part of the nation's center of tornado activity which is in Kansas, Oklahoma, and Texas (Grazulis, 1993). Yet, Illinois has experienced the nation's two longest track and most damaging tornadoes (Fujita, 1987). The first in 1917 covered 293 miles and the second in 1925 covered 219 miles, and both had wide tracks that varied from 1/4 to 1 mile wide. The average size of a tornado in Illinois has a length of 14 miles and a width of 1/16 mile (Wilson and Changnon, 1971). Although aerial photography was not available to do post-storm assessments in 1917 or 1925, detailed field studies of both storms by weather scientists and engineering groups gave proof the storms were continuously touching the ground, resulting in the record distances.

The two long track tornadoes had near record forward speeds, 40 mph in 1917 and 62 mph in 1925. Both storms were extremely deadly and damaging. Their large funnel sizes, high-speeds, and long path lengths from beginning to end were factors leading to many deaths and large damages. Illinois normally experiences only three tornado deaths each year (Changnon and Kunkel, 2006). Property losses in 1917 amounted to $8.6 million (1917 dollars) and those in 1925 amounted to $24.4 million (1925 dollars). The 1917 losses expressed in 2008 dollars are $51.5 million, while the 1925 losses are equivalent to $134.2 million in 2008 dollars.

The two storms occurred at a time when there was no scientific understanding of atmospheric conditions that formed tornadoes; hence, no forecast skills existed. Further, there was no detection nor warning system in place. Tornadoes were detected solely by visual sightings of the funnel.

1917 MATTOON TORNADO

The funnel of the tornado on May 26, 1917 first appeared at noon just west of a small town southeast of Quincy (Wilson and Changnon, 1971). It moved east along a remarkably straight line creating damages in several small towns (Fig. 1) and many farms. At times along its path the tornado was accompanied by large hailfalls, with hailstone diameters of 3 inches. The tornado then struck and badly damaged both Mattoon and Charleston.

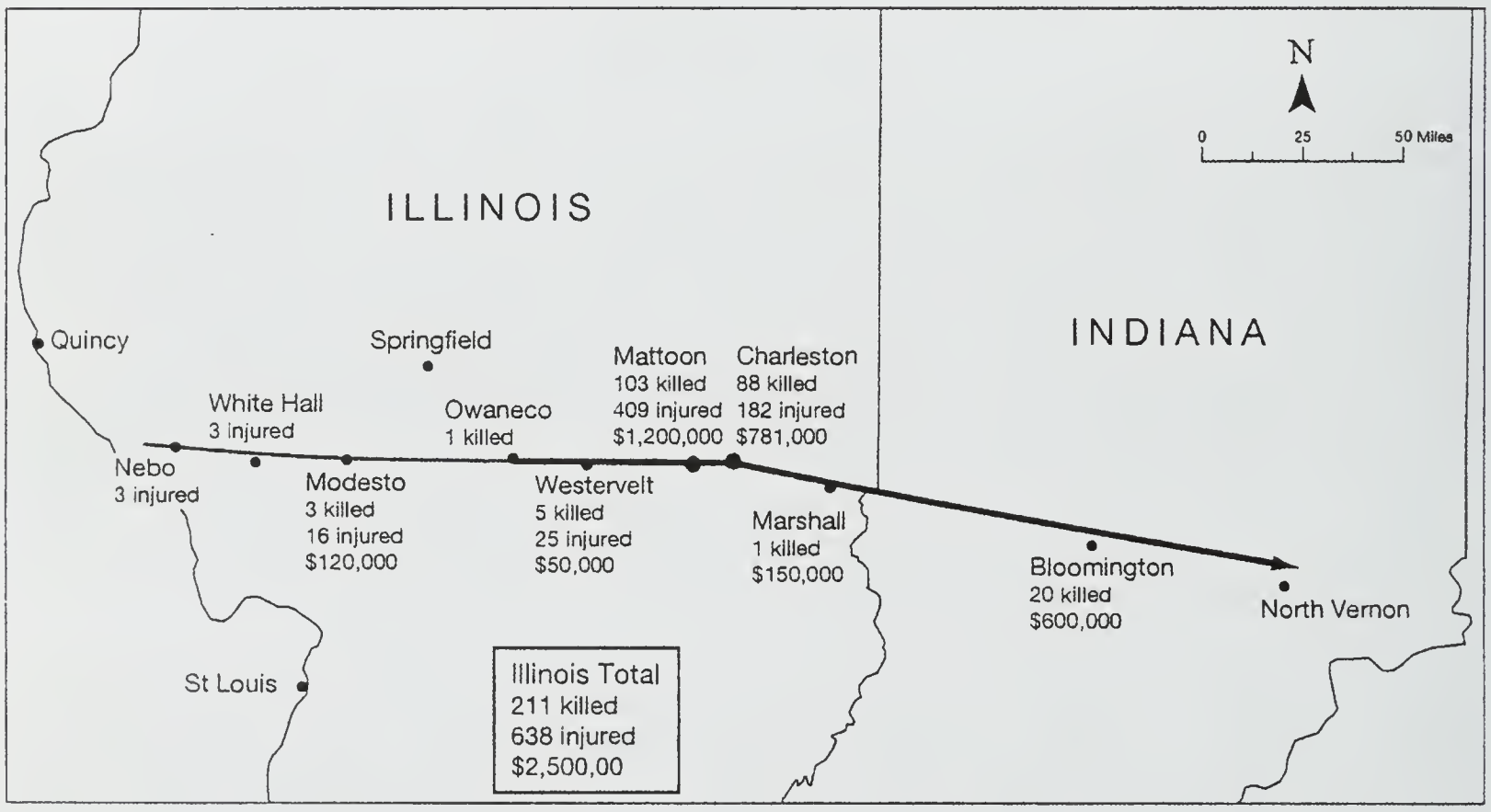

Figure 1. The track of the Mattoon tornado on May 26, 1917. The track was 1/4 mile wide from Nebo to Owaneco, and then expanded to ½ mile wide.

In Mattoon an area 2.5 city blocks wide and 3 miles long was totally devastated. The destruction included 103 persons killed, 400 injured , and 496 houses were totally destroyed, leaving 2,500 residents homeless. The local damages totaled $4.2 million

(1917 dollars). These huge losses led to this tornado becoming named the "Mattoon Tornado." The storm was also very damaging to buildings in Charleston. There it passed through the business district causing great damage to commercial interests. In Charleston the storm killed 88 persons, injured 182, destroyed 221 homes, and left 385 persons homeless. The total local damages were $3.7 million. Other damages occurred in several small communities in Illinois (Fig. 1).

Persons in the Mattoon and Charleston area reported never seeing a funnel, just a large dark cloud reaching the ground. Hence, no one suspected a large and dangerous tornado was approaching. After passing through Charleston, the storm's track shifted to the east-southeast and it entered Indiana after a 188-mile trip across Illinois. The storm crossed three-fourths of Indiana and terminated near North Vernon, having a duration of 7 hours and 20 minutes. The tornado averaged 0.5 mile wide after reaching Owaneco (Fig. 1). Indiana had 20 citizens killed and damages that totaled $0.6 million (1917 dollars). Summation of the 2-state total damages shows that 231 persons were killed, 638 were injured, and property losses amounted to $8.6 million (1917 dollars).

1925 TRI-STATE TORNADO

The second long-track tornado in Illinois occurred eight years later on March 18, 1925. This tornado ranks above all other 16,730 tornadoes in the nation since 1915 as the single most devastating storm (Grazulis, 1993). After it occurred, it became labeled as the Tri-State Tornado (Changnon and Semonin, 1966). It began at 1 pm in southeastern Missouri, lasted 3.5 hours, and covered 219 miles across southern Illinois and into southwestern Indiana (Fig. 2). The resulting losses included 695 dead, 2,027 injured, and damages of $24.4 million (1925 dollars). This is the greatest death total ever from a tornado in the U.S. (the next highest is 306), and its losses rank as the nation's second largest financial loss from a tornado (Grazulis, 1993). The significant climatological aspects of the storm include: 1) second longest tornado on record, 2) near record fastest speed of movement (62 mph), and 3) unchanging production of extreme winds throughout most of its 3.5-hour duration. An important aspect of this tornado was the extreme width of its path. In Missouri the path was 1/4 mile wide, but in Illinois it became a mile wide and varied from ½ to 1 mile wide in Indiana (Fig. 2). Its forward speed of 62 mph ranks as the third fastest moving tornado ever.

Another important aspect of the tornado was the lack of a visible funnel, the cause of many deaths. In Missouri a large funnel was seen, but in Illinois none was apparent. In Indiana, a funnel became visible. The inability to see a funnel in Illinois was a result of its great width (½ to 1 mile) and the large volume of dust and debris in the air. To most viewers in Illinois the storm appeared to be a large and dark approaching thunderstorm. Hence, few sought tornado shelters.

The storm produced most of the damages during a 40-minute period as it moved from Gorham to Parrish, Illinois. In a 47-mile distance it killed 541 persons, injured 1,423, and produced $18.3 million in damages (1925 dollars). Building destruction in Murphysboro and West Frankfort was extensive and mainly in residential sections. In addition, tipples and buildings at five coal mines were badly damaged. The remainder of the storm's track across Illinois was largely through rural farmland with fewer major damages. The storm

struck the industrial section of Princeton, Indiana, resulting in 45 deaths and over $3.9 million (19925 dollars) in property losses. The large number of injured persons in Illinois could not be handled in local hospitals in Murphysboro and West Frankfort. As a result, hundreds of injured persons were shipped by special hospital trains to other hospitals in southern Illinois and Missouri (Akin, 2002).

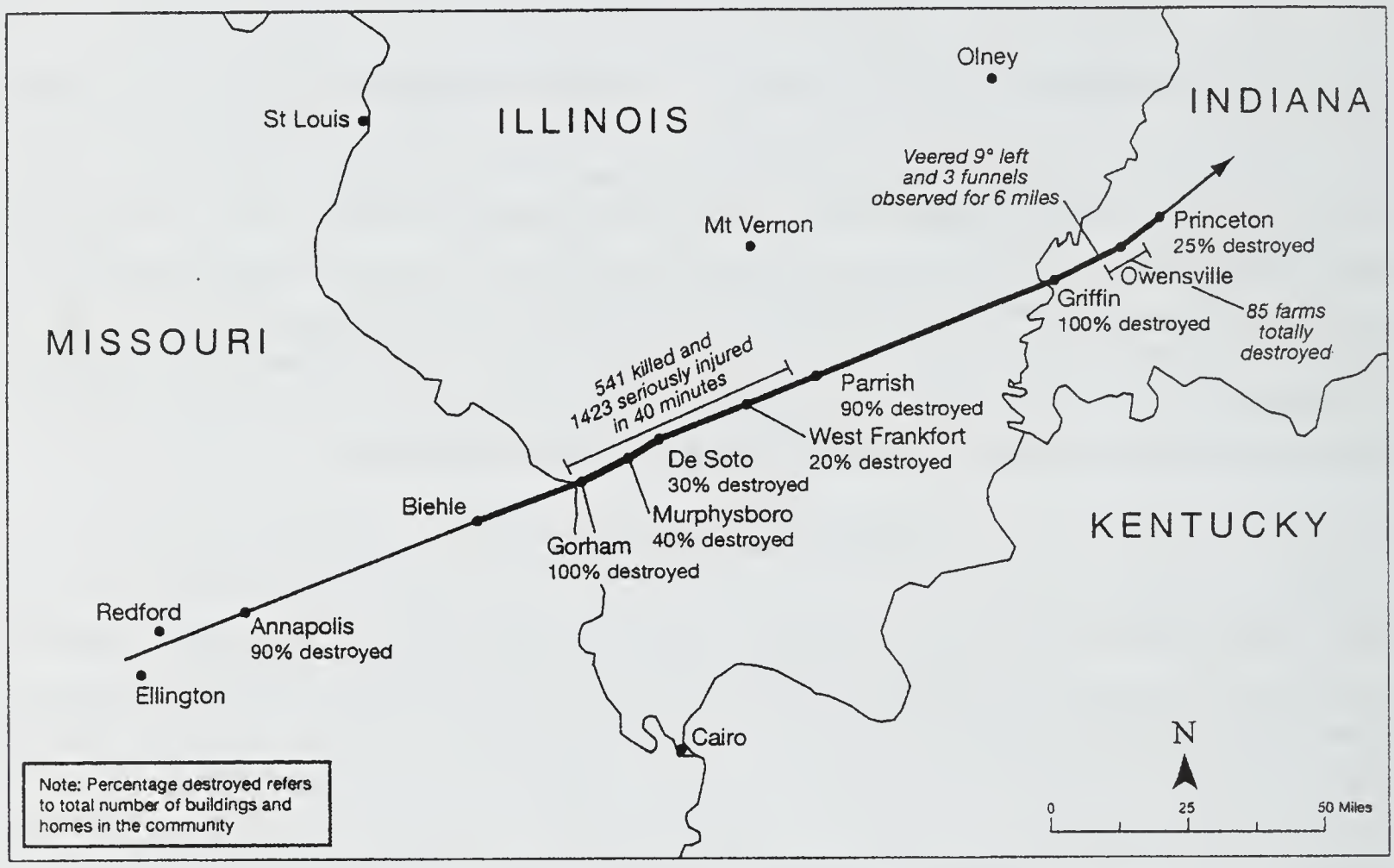

Figure 2. The track of the Tri-state tornado on March18, 1925. The path up to Biehle, Missouri, was 1/4 mile wide, then expanded to 1 mile wide, decreasing to ½ mile at Princeton, Indiana.

The storm's large loss of life and massive damages led an engineering society to assemble a team of engineers to survey the damages along the 219-mile path of the storm (Young et al., 1925). Their 1925 storm survey identified the primary factors leading to deaths and damages: 1) no tornado forecast, 2) no warnings of an approaching storm, 3) exceptionally fast forward speed, 4) unusually large storm size, 5) high wind speeds, 6) lack of adequate shelters, 7) lack of a tornado appearance, and 8) poor construction techniques in existing homes and structures. Many deaths and considerable property damage, aside from those due to the collapse of buildings when hit by the tornado funnel, were caused by flying debris, often of exceptional size. For example, walls of houses and roofs of barns were lifted aloft and then blown around by the big funnel.

POST STORM IMPACTS AND LESSONS LEARNED

The two tornadoes resulted in long-lasting and important impacts. These included effects on the Illinois public and their behavior, lessons about building design and construction requirements, and information for the scientific community.

The storms had long lasting impacts on the behavior of local citizens. Several decades after the Tri-State storm, persons in Murphysboro and West Frankfort were still going to shelters when a dark storm cloud appeared (Akin, 2002). Many did this even when the National Weather Service had not issued a tornado watch and when no local warnings were issued on the radio nor sirens sounded. Local memories of the Tri-State Tornado were revealed by local responses to a hailstorm in 1968, 43 years after the tornado. This damaging hailstorm had a 25-mile track along a portion of the Tri-State Tornado track in southern Illinois, and the coincidence of the hailstorm and famed tornado track was widely publicized by the regional news media (Changnon, 1970).

Another action widely noted in and around the storm areas was the construction of hundreds of storm shelters. People dug underground spaces large enough to hold their families so as to hide from falling walls or flying debris. A second result that was partially prompted by the Tri-State Tornado of 1925 was a change in Midwestern property insurance coverage. In 1930 the industry broadened existing property coverage in the Midwest to include "tornado insurance", something that had not been covered before (Kunreuther, 1998).

One positive outcome stemming directly from the tornadoes came as a result of the report issued by the engineering team that surveyed the Tri-State Tornado (Young et al., 1925). Their report noted many structural failures and recommended major changes in home and building construction techniques. These were adopted as local and state rules that led to improved and stronger construction techniques used in future house and business structures.

The storms also had long-term impacts on the scientific community. These two storms were an integral part of studies of thunderstorms (Byers and Braham, 1948). Later studies assessed long-track tornado formation and persistence. The advent of weather radars in World War II allowed post-war studies of tornadoes that produced long-track tornadoes. The first long-track tornado (covering 68 miles) detected and monitored by radar occurred in east-central Illinois on April 9, 1953 (Stout and Huff, 1953). Ensuing storm research discovered that a few thunderstorms producing long-track tornadoes develop in areas with unique atmospheric circulation patterns aloft; these have subsequently become labeled as "supercells" (Rauber et al., 2002). The two record long tornadoes of 1917 and 1925 and their record high losses have had major impacts on weather research. The price that hundreds of Illinois citizens paid nearly a hundred years ago has helped lead to the saving of thousands of lives in ensuing years.

LITERATURE CITED

Akin, W., 2002: The Forgotten Storm: The Great Tri-state Tornado of 1925. Lyons Press, Guilford, CT, 172 pp.

Byers, H.R., and R. Braham, 1947: The Thunderstorm. U.S. Weather Bureau, Washington, DC, 112 pp.

Changnon, S.A., 1970: Major hailstorms retrace Tri-state tornado track in Illinois. Transactions Illinois Academy of Science, 63, 34-41.

Changnon, S.A., and R. G. Semonin, 1966: A great tornado disaster in retrospect. Weatherwise, 25, 56-65.

Changnon, S.A., and K.Kunkel, 2006: Severe Storms in the Midwest. Illinois State Water Survey, Champaign, IL, 74 pp.

Fujita, T.L., 1987: U. S. Tornadoes, Part 1. SMRP Paper 218, Department of Meteorology, University of Chicago, Chicago, 218 pp.

Grazulis, T.P., 1993: Significant Tornadoes: 1680-1989. Environmental Films, Johnsburg, VT, 245 pp.

Kunreuther, H., Instability conditions and the supply of coverage. Paying the Price. National Academy of Science, Washington, DC, 17-50.

Rauber, R.M., Walsh, J., and D.J. Charlevoix, 2002: Severe and hazardous weather. Kendall/Hunt Publishing Co., Dubuque, IA, 615 pp.

Stout, G., and F.A. Huff, 1953: Radar records Illinois tornado, Bulletin American Meteorological Society, 34, 16-25.

Wilson, J., and S.A. Changnon, 1971: Illinois Tornadoes. Illinois State Water Survey, Champaign, Illinois, 58 pp.

Young, H. Lowenberg, M., Randall, F., Shaub, J., and F. Vent, 1925: Report on effects of tornado of March 18, 1925, Journal of the Western Society of Engineers, 30, 373-396.

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Impacts of the 2008 Floods on Railroads in Illinois and Adjacent States

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ABSTRACT

Near record floods in the Midwest during the spring-summer of 2008 brought massive damages to railroads and train movement problems. Illinois is in the busiest rail area of the nation. All seven of the nation's seven major railroads experienced losses. The damages led to closures of 56rail lines in the 5-state area centered on Illinois, and many closures lasted a week or longer. Many passengers could not travel on Amtrak trains since many were cancelled for weeks The damaged tracks led to eight train wrecks and nine bridges were ruined. The damage costs to the 15 railroads affected were $85 million; the revenues lost totaled $69 million; and the total of $154 ,million rates as one of the major rail losses on record.

Key Words: floods, railroads, Midwest, heavy rains, damages

INTRODUCTION

The Midwest experienced a stormy and wet winter from December 2007 to February 2008. Then the February-April period was the wettest on record for the Midwest, averaging 39 cm of precipitation across the region. This saturated the soils. Beginning in March, and continuing through June, there were several major rainstorms that occurred in the Illinois, Indiana, Iowa, Missouri, and Wisconsin area. These events created sizable floods on the small and large rivers of this 5-state area.

This severe flooding produced major problems for the areas numerous high volume railroads. Prior studies have shown that flooding hurts the rail industry much more than other forms of surface transportation because they have more spatial flexibility than railroads (Changnon, 2006). The 2008 floods washed out rail roadbeds, buried rail lines underwater, and destroyed several rail bridges, leading to 56 line closures that lasted anywhere from 2 to 48 days. Trains were halted and hundreds had to take long, costly detours over other non-flooded railroads. Travelers on numerous key Amtrak trains could not get served due to numerous train cancellations resulting from flooded tracks and closed lines.

Atmospheric sciences research in recent years has emphasized assessments about how weather affects the environment, society, and the economy. A recent book published by the American Meteorological Society addressed the weather impacts on U.S. railroads (Changnon 2006). This study assesses how varying rain conditions created different kinds of floods and damaged railroads.

Floods in the 5-state area were financially devastating to the railroads because this area has a dense network of very busy rail lines. The nation's three busiest rail centers are Chicago, Kansas City, and St. Louis, and the heavy rains and ensuing flooding occurred in the area between these centers where rail traffic is exceptionally frequent and valuable. At times, trains were trapped and had to wait days before they could be moved. Several busy mainlines were closed for days and weeks. Eight trains experienced accidents related to the flooding. Most of the region's railroads cooperated and allowed trains of a closed railroad line to detour onto their non-flooded lines to get around the flooded area. The railroads most impacted by the 2008 flooding were in Illinois and Iowa where rail-flood problems occurred in most parts of both states. The lines in the flooded area are shown on figure 1, and the line closures are marked, including the number of days the line was closed.

The costs to the railroads included loss of business and penalties for late shipments. Costs also included those related to recovery actions, such as the laying of new roadbeds and track, bridge rebuilding, and car and engine repairs. Train crews often became a problem since the detours required much additional time and thus additional crews. Detours were circuitous and costly due to the greater distances traveled, added train crews, and the high costs of fuel in 2008. The floods even damaged valuable rail museums. For example, the Mid-Continent Railway Museum in Wisconsin was flooded under several feet of water when the Baraboo River overflowed and created much damage to its facilities (Carstens, 2008).

The damages and operational problems created for each of the 15 railroads impacted by the flood are described. Then, the region's problems, responses, and costs are reviewed.

HEAVY RAINS AND FLOODING

A heavy rainstorm in mid-March produced 25 to 33 cm of rain in southeastern Missouri and southern Illinois, following a winter with above normal precipitation in Illinois, Missouri, Iowa, and Wisconsin (Changnon et al., 2008). Rainfall in April from southern Missouri to the eastern half of Iowa and into southern Wisconsin was 150 to 200 percent of normal. Heavy rains, 6 to 13 cm, fell on April 3-4 across southern Missouri and southern Illinois and created several flash floods. Another major rainstorm on April 9-10 (8 to 10 cm) fell across northeastern Missouri and southeastern Iowa. In May, heavy rains again fell across Iowa, Illinois, Missouri, and Indiana. Monthly amounts were 150 to 200 percent of normal.

June was also very wet with monthly amounts greater than 200 percent of normal across Iowa, Missouri, southern Wisconsin, south-central Indiana, and central Illinois (MRCC, 2008). Many rain totals exceeded 30 cm in central-east Iowa, south-central Indiana, and northern Missouri. Elsewhere totals ranged from 20 to 26 cm. One Indiana weather sta-

tion had 51 cm in June, double the previous record. July rainfall was 150 percent of normal in a small area of central Iowa and northeastern Missouri (MRCC, 2008). Elsewhere in the 5-state area with floods, the July rainfall was near normal with less than normal in the northern half of Wisconsin and across Indiana. August had below normal rainfall throughout the 5-state area.

The result of the extremely wet winter and spring 2008 was devastating flooding in Iowa, southern Wisconsin, southern Indiana, southern and western Illinois, and northern Missouri. In Iowa, record crests occurred on the Cedar River and several other tributaries of the Mississippi River. By late April, the Mississippi River had exceeded its past record highs set in 1973 in the Arkansas-Mississippi area (Natural Hazards Observer, 2008).

At the end of June, streamflows of many rivers in western Illinois, eastern Iowa, northern Missouri, and southern Wisconsin were 190 to 200 percent of normal levels. Flood waters spread over many lands, and the occasional intense rainstorms brought several flash floods in the 5-state area. Flooding extended into July but began to rapidly diminish after mid-July. The year's highest levels on the Mississippi River from Dubuque south to Cairo (Fig. 1) occurred during the July 1-5 period, and then fell to more normal levels by the end of July (Scott, 2008).

PROBLEMS EXPERIENCED BY RAILROADS

Railroads are classified into three classes according to their income and size. The nation has seven "major railroads," each a Class 1, with earnings greater than $90 million annually and with thousands of kilometers of tracks extending across multi-state areas. These seven include the Burlington Northern Santa Fe, Canadian National, Canadian Pacific, CSX, Kansas City Southern, Norfolk Southern, and Union Pacific. Class 2 railroads are also labeled as "regional," and these railroads have incomes between $40 and $90 million annually with more than 570 km of tracks. They typically have rail lines in 1 or 2 states. The Class 3 railroads have annual incomes less than $40 million with trackage less than 160 km and often are labeled as "short Lines." These typically serve local industries like grain elevators, and act to feed freight shipments to the larger railroads. The impacts of the flooding are presented for each railroad according to these three classes, plus those experienced by Amtrak.

Amtrak is a government-sponsored railroad that operates passenger trains over the lines of the seven major railroads. Amtrak service was seriously affected in 2008 by line closures on its host routes (Lamkenau, 2008). Severe flooding in southern Illinois, south of Carbondale (Fig. 1) in March, closed the CN mainline. Amtrak stopped the *City of New Orleans* at Carbondale on March 19, and all passengers were bussed to Memphis. Busses brought northbound passengers from Memphis to Carbondale. This line was re-opened on March 25, and the train resumed operations between Chicago and New Orleans on March 26.

Because of early June flooding on the east-west BNSF mainline in Iowa (Fig. 1), the *California Zephyr* did not run east of Denver beginning on June 13, but resumed operation on July 7. The *Southwest Chief* was suspended on the BNSF line between Chicago and Kansas City from June 17 until July 1 due to flooding. The *Empire Builder's* route

over the Canadian Pacific (CP) from Chicago to St. Paul was disrupted by flooding in southern Wisconsin. The train was suspended on June 10, and resumed operations on June 27 by using a detour on the UP line from Chicago to Milwaukee and on to La Crosse.

Major Class One Railroads

The *Burlington Northern Santa Fe* (BNSF) was the hardest hit of the seven major railroads. This is the nation's largest railroad and has three heavily trafficked main lines in the 5-state area. The mainline between Denver and Chicago, which runs west-east across Iowa and Illinois, was submerged at several locations (Fig. 1) and was closed for 7 days near Ottumwa. In Illinois, a levee along the Mississippi River broke and flooding submerged this main line for 16 days. The busy BNSF line between the Twin Cities and Chicago was closed for several days at two flooded locations along the Mississippi River in Wisconsin (Fig.1). The BNSF and CN also closed their flooded parallel lines from Dubuque to Portage, Illinois.

BNSF's very busy transcontinental route between Chicago and the West Coast was threatened at Ft. Madison (Fig.1). To avert closure, the BNSF used thousands of tons of gravel to raise 17 miles of the main line by 1 to 2 feet alongside the Mississippi River (Lustig, 2008). However, a large washout occurred in Missouri and closed this line for three days in June. Sections of the BNSF lines north and south of St. Louis were also closed, often because flood gates in several urban locations were closed by the U.S. Corps of Engineers. The BNSF line south of St. Louis and along the Mississippi River was flooded and closed during March, and then closed again until July 7 when flood gates at Cape Girardeau were closed.

The *Canadian National* (CN), owner of the former Illinois Central lines in the 5-state area, had two busy mainlines seriously damaged by the floods. One extends from Chicago to New Orleans, and the other goes west from Chicago to Omaha. Heavy March rains across southern Illinois created flooding that closed both of CN's north-south lines there (Fig. 1). Most flooding was a result of a March rainstorm that put down 28 to 33 cm of rain. Water 30 cm deep covered the CN mainline south of Carbondale (Fig. 1), and a 2-meter deep washout left the track suspended in mid-air for several hundred meters near Makanda. This stopped all trains and a grain train was stranded. All other freight traffic was shifted to the CN's other north-south line from near Effingham into Kentucky (Fig.1). Then, heavy rains and flooding closed this line in two locations; thus, both CN north-south lines were closed and all trains were stopped for three days.

Severe rainstorms in early June in Iowa hurt the CN. Several sections of its west-east line were out of service, as shown in figure 1. Trees fell across the lines, high waters threatened bridges, and many tracks were flooded. The line in western Iowa was flooded with water going over a bridge near Dennison, and a 730-meter section of the bridge was destroyed. As a result, the Fort Dodge–Omaha section of this line was closed for 8 days. Water was also over this CN line in central Iowa and tracks were buried by a major mud slide near Cedar Falls. Ten rail cars were put on a bridge near Cedar Falls to try to stabilize it. The line west from Dubuque to Cedar Falls was closed for 8 days. The BNSF and CN closed their flooded parallel lines from Dubuque to Portage, Illinois. This trapped

eight CN trains in Dubuque. The CN mainline in central Wisconsin was closed on June 8, and all trains were held until waters receded on June 11.

The *Canadian Pacific's* (CP) mainline between La Crosse and Chicago was closed in central Wisconsin when one meter of water covered the rails. CP trains were detoured over several routes including Wisconsin & Southern (WS) between Madison and northern Illinois. Service on the CP line was restored 19 days later. A detouring CP train on the IC&E derailed in northern Illinois on June 24, closing the busy IC&E line between Chicago and Savanna for two days.

In the 5-state area the *CSX Railroad* has busy lines extending from Chicago south and an east-west main from St. Louis east to Indianapolis and on to many East Coast locations. The north-south mainline became flooded near Beecher, Illinois, closing the shared mainlines of CSX and UP on March 26. The flooding led to the derailment of 22 cars of a UP freight, and this further blocked the line for 4 days. CSX trains were detoured to the east. The CSX mainline between Indianapolis and St. Louis was closed at Greencastle, Indiana by a washout on June 8. The line was opened on June 12, and 28 stalled trains resumed operations..

The *Kansas City Southern* (KCS) lines served to handle many train detours. Flooding in Missouri, Arkansas, and Mississippi during April and May affected many train operations leading to detours over the KCS lines. Heavy rains occurred across KCS territory with minor flooding in western Missouri, and KCS trains south of Kansas City had to move very slowly for 20 days. Flooding covered the rail line along the Mississippi River at Louisiana, Missouri, and led to closure for two weeks of the KCS line to St. Louis.

An eastbound freight train on the *Norfolk Southern* (NS), going from Kansas City to Decatur, derailed 32 cars east of Hannibal (Fig. 1) on June 3, a result of the failure of a flooded bridge (Railroad Illustrated, 2008). The flood waters also brought debris onto the line but the line was reopened two days later. The Corps of Engineers closed the flood gates at Hannibal, Missouri, on June 13, closing the busy Decatur-Kansas City line for 21 days, a major problem since this line carries much freight from the West Coast to East Coast sites. Flooding from levee failures also eroded the line near Hannibal.

Flood troubles on the *Union Pacific* (UP) began early. The UP has three main lines in the 5-state area, plus several other lines once owned by the C&NW Railroad. A flash flood in southern Illinois in March washed out the roadbed under 85 meters of UP line near West Vienna. Seven cars of a passing UP train derailed, and two crewmen were injured. UP's main line across Iowa and Illinois_handles 70 trains each day, and it experienced numerous problems including closure when the Cedar River bridge was threatened. The line west of Cedar Rapids was closed for 3 days, and the UP closed this mainline again when the bridge across the Cedar River at Waterloo collapsed on June 10. The UP closed the Chicago-Omaha mainline near Cedar Rapids for 6 days and the rail yards there were flooded. The UP had 175 trains held awaiting the line's opening, and after it was opened, it took three days to clear this mass of stalled trains (Lustig, 2008) The UP's mainline between St. Paul and Chicago was closed in Wisconsin (Fig. 1) by floods on June 8.

Regional Class Two Railroads

In April many trains of the *Iowa, Chicago & Eastern* (IC&E) had to detour due to the floods in Iowa. High waters destroyed a bridge south of Marquette, Iowa. Due to levee failures, the Mississippi River flooded and closed the IC&E main line at Davenport on April 26. IC&E trains going to and from Chicago were detoured onto the CP line. In late April, IC&E trains from the Twin Cities (MN) to Kansas City were shifted to the UP line from Mason City to Kansas City (Fig. 1). The IC&E line from Davenport to Kansas City was closed for 7 days at a site near Davenport, and for 35 days at another badly flooded location. The CP handled 46 detoured IC&E trains going to Chicago, and the UP handled 29 IC&E trains from Kansas City to Des Moines.

Heavy rains in northeastern Iowa on June 7-8 washed out the IC&E line for 3 miles between Marquette and Mason City (Fig.1). A bridge on this line was washed away and this line was closed for 12 days north of Clinton. A train derailed south of Marquette on July 9, 30 cars derailed, and four engines fell into the Mississippi River causing serious injuries to the crew. The Rock River flooded the IC&E line between Rockford and southern Wisconsin for 6 days.

Several railroads used the IC&E for detouring trains around floods. UP trains used the line between Clinton and Kansas City from June 7 to 10. UP coal trains going east from Kansas City used the IC&E at Kansas City to get across Iowa before the line closed. The IC&E handled some BNSF detours from Savanna to Clinton. The IC&E bridge over the Iowa River at Columbus Junction collapsed on June 25, dumping the engines of a passing freight train into the river and injuring the crew.

The *Iowa Interstate* (IAIS) operates an east-west main line from Omaha east to Chicago, plus several branch lines in both states. The IAIS had several lines in Iowa closed by floods in early June. A branch line at Des Moines was closed 18 days by washouts. The UP put a train on IAIS mainline from Omaha to Des Moines on June 8, but it was s stopped by a major washout that closed the line for ten days. A short line, the Cedar Rapids and Iowa City Railway (CIC), closed its damaged bridge over the Iowa River near Amana, cutting off IAIS access to Cedar Rapids for 23 days. Several Iowa City-based trains, which normally ran to Cedar Rapids (north) and to Rock Island (east), were stopped. The IAIS line between Davenport and Iowa City was closed for 13 days (Fig. 1), and a train near Davenport hit a washout and was wrecked on June 13.

The *Wisconsin and Southern* (WS) has four lines that radiate out of Madison, and three were closed for more than 30 days. The line south from Madison, which had been hosting detoured CP and UP trains, had to be closed for 2 days.

Class Three Short Lines

The Cedar River in Iowa, which crested at record levels in June, wiped out the bridge of the *Cedar Rapids & Iowa City* (CIC) at Cedar Rapids on June 12. The bridge was also used by the IAIS, and was closed 23 days.

The June floods created major havoc for the *Iowa Northern* (IANR). Its rail car shops were flooded at Waterloo, just southeast of Cedar Falls (Fig.1), but the IANR managed to get all stored rail cars moved out of town. IANR had several washouts north of Cedar

Rapids, and a 2-mile one near Mason City closed the line for 14 days. The UP's destroyed bridge near Cedar Rapids severed the CIC and IANR lines which also used this bridge. The city of Waterloo closed its flood gates on June 10, thus closing the IANR route south to Cedar Rapids for 24 days.

The *Louisville & Indiana* (L&I) line between Indianapolis and Louisville was closed when a bridge was washed away on June 8. The line was re-opened 23 days later when the bridge was re-built.

The *Indiana Railroad* (IRR) line to Newton, Illinois, had multiple washouts and a washed out bridge, leading to its closure for 6 days. The Terre Haute-Bedford line also had many washouts, being closed for 7 days. The line near Indianapolis had major washouts and was closed for 48 days.

The *Peoria and Western* line from Peoria west to Keokuk, Iowa, was closed for 29 days. The bridge over the Mississippi River at Keokuk was considered unsafe to cross due to high water and swift currents.

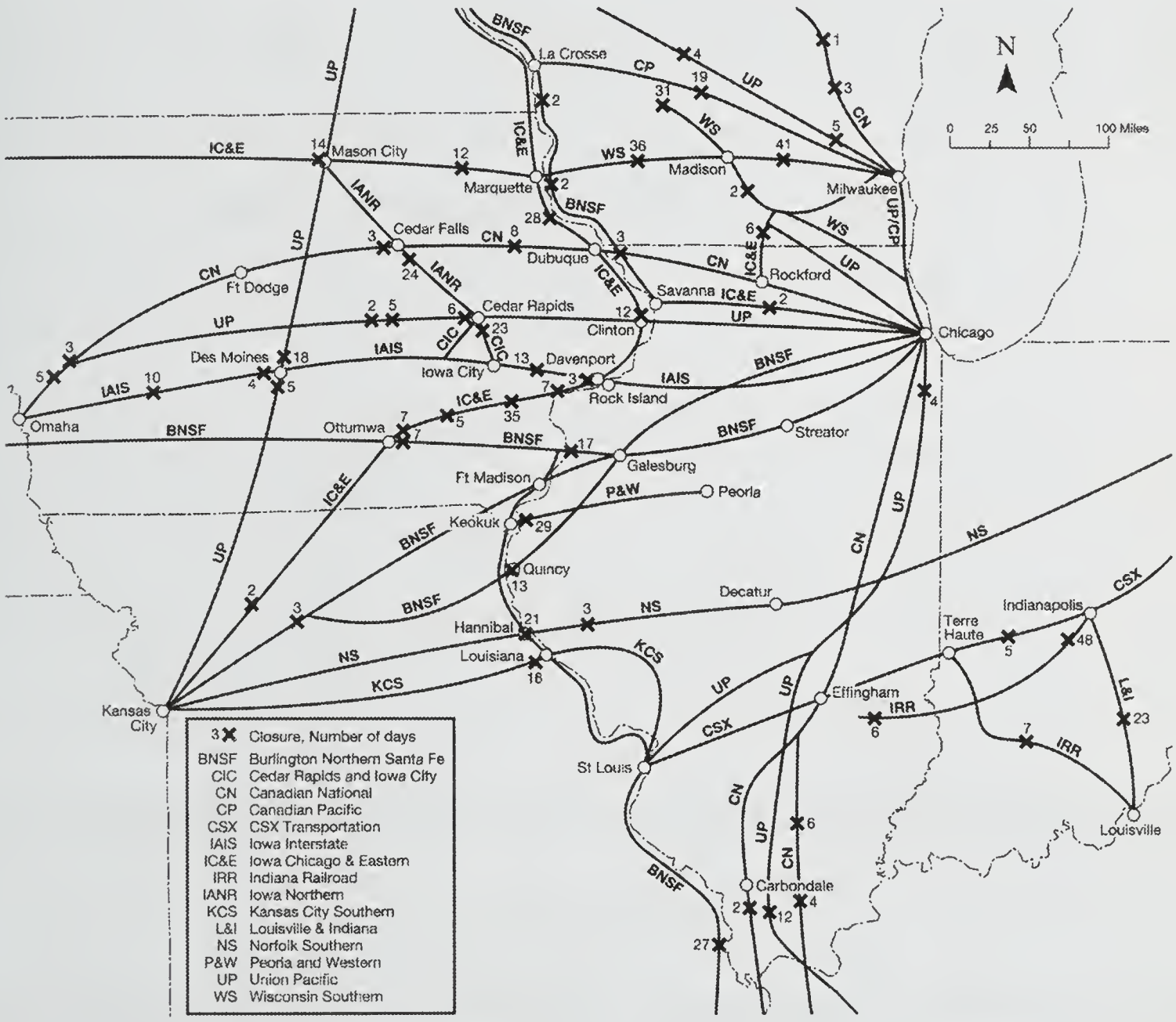

Figure 1. Rail lines in the flooded area, and locations of line closures are shown along with the number of days closed.

ECONOMIC IMPACTS

Flood-related damages to tracks, roadbeds, bridges, signals, and other railroad facilities were excessive, totaling $85 million in costs to repair. The 2008 revenue losses also were quite high, $69 million. The total losses and costs was $154 million. This was high but much less than those caused by the record high waters and long lasting 1993 Midwestern floods, which caused railroad losses and costs amounting to $495 million, the highest ever experienced in the U.S. (Changnon, 1996). Replacement of washed-out rail lines and roadbeds cost $1.2 million per mile, and there were 31 such miles costing $37 million to repair. The costs to repair lines that were under water averaged $130,000 per mile, and with 134 miles under water, the total cost to repair them was $17.4 million.

Table 1 shows the 2008 damages experienced by each railroad. The BNSF had the greatest damages, $26 million. Also shown in the table are losses in income due to the floods. The BNSF and UP had the greatest revenue losses. The state of Iowa's Railway Finance Authority provided $4 million to the regional and local railroads to help restore their damaged lines in Iowa.

Table 1. The economic impacts of the floods of 2008, and the values shown are millions of dollars.

Railroad	Damage Costs	Revenue Losses
Amtrak	2	3
BNSF	26	9
CN	17	10
CP	4	3
CSX	3	2
IC&E	10	6
IAIS	3	3
KCS	1	3
NS	1	4
UP	14	15
WS	2	1
Short Lines (4)	2	1
Total	**85**	**69**

SUMMARY

The railroad problems caused by the 2008 floods, and the responses the railroads used to address the problems are listed in table 2. The problems included both damages and losses in revenue due to trapped trains and delayed shipments. Rainfall during January-July 2008 exceeded 65 cm in Illinois, Indiana, Iowa, and Missouri, and amounts ranked

as the highest on record. The numerous heavy rain events led to numerous severe floods of near record proportions.

Table 2. The problems and responses used by the railroads to deal with the 2008 flood problems.

Problems	Responses
washed out lines	detours of trains
submerged tracks and rail yards	repairs of track and roadbeds
damaged/destroyed bridges	bridges rebuilt
lines closed for days	passengers bussed
trains trapped	repaired rail cars
shipments delayed	rebuilt railroad facilities

Flooding along the Mississippi River was a major problem for the railroads since so many lines cross the river and/or are built parallel to the river. Below is a list of the thirteen locations where rail lines were closed as a result of flooding along the Mississippi River, listed from north to south sites.

- La Crosse, WI—BNSF
- Prairie du Chien, WI—BNSF
- Guttenberg, IA—IC&E
- Portage, IL—BNSF and CN
- Clinton, IA—IC&E
- Davenport, IA—IC&E
- Burlington, IA—BNSF
- Ft. Madison, IA—BNSF
- Keokuk, IA—P&W
- Quincy, IL—BNSF
- Hannibal, MO—NS
- Louisiana, MO—KCS
- Cape Girardeau, MO—BNSF

This list reveals that the BNSF was stopped at 7 of the 13 locations, further revealing why the BNSF suffered the most losses from the 2008 floods. In the flooded area, the BNSF had the greatest mileage of heavily traveled mainlines between the Midwest and the West Coast. The railroads crossing the Mississippi River on bridges between the Quad cities and St. Louis all had serious problems with the bridges and their approaches across adjacent flood plains. The bridges included, from north to south, the ones at Burlington, Ft. Madison, Keokuk, Quincy, Hannibal, and Louisiana. Flooding in this 5-state area was a major problem for all the nation's seven largest railroads.

There were nine rail bridges destroyed or seriously damaged. Their state locations are listed in table 3, revealing that six occurred in Iowa. Also listed in the table are the number of train wrecks from the flood. Notably, four of the eight train wrecks were in Illinois. The number of line closures reveal that Iowa led with 23, followed by Wisconsin and Illinois each with 12 lines closed. The 56 closures totaled 640 days, an average of 11 days per closure.

Table 3. Number of rail bridges destroyed or badly damaged, the number of train wrecks, and number of rail line closures due to the 2008 flood.

States	Bridges	Train Wrecks	Line closures
Illinois	2	4	12
Indiana	1	0	4
Iowa	6	3	24
Missouri	0	0	5
Wisconsin	0	1	12
Total	9	8	56

Many problems and major costs were experienced by the Midwest's railroads in dealing with the 2008 floods. However, regional railroad experts noted that the flooding was not as severe as experienced in 1993. The economic impact of the 2008 floods totaled $154 million, roughly a third of the record losses/costs of $495 million resulting from the 1993 floods that occurred in the same general area (Changnon, 1996).

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LITERATURE CITED

Carstens, H., 2008: Floods Ravage Rail Museum. Railfan and Railroad, 27, 4.

Changnon, S.A., 1996: Impacts on Transportation Systems: Stalled barges. Blocked Railroads, and Closed Highways. Great Flood of 1993, Westview Press, Boulder, CO, 183-204

Changnon, S.A., 2006: Railroads and Weather. American Meteorological Society, Boston, MA., 87pp.

Changnon, S.A., Kunkel, K., and D. Changnon, 2008: Winter 2007-2008: Record-setting Storms Caused Major Damages in Illinois. Illinois State Water Survey, Data/Case Study 2008-2,, 41pp.

Lamkenau, W.C., 2008: Flooding Devastates Midwest Railroads. Railfan and Railroad, 27, 26-27.

Lustig, D., 2008: Floods Devastate Midwest Routes. Trains, 68, 8-9.

Midwest Regional Climate Center, 2008: Climate Summary for July. Champaign, IL

Natural Hazards Observer, April 2008: Highest Flood Level on Lower Mississippi since 1973. 9-10.

Railroads Illustrated, June 2008: Midwest Storms Hit Iowa Lines. 10-15.

Scott, R., 2008: Illinois Water and Climate Summary for July 2008. Illinois State Water Survey, 12pp.

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Turtles of Bond, Macoupin and Montgomery Counties, Illinois, 2006-2008

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ABSTRACT

During 2006-2008, we used baited hoop nets to sample Bond, Macoupin and Montgomery counties for the presence of aquatic turtles. We had 457 captures of turtles representing five species (*Chelydra serpentina*, *Chrysemys picta*, *Trachemys scripta elegans*, *Sternotherus odoratus* and *Apalone spinifera*) during 122 net-nights of effort at 53 sites. Lentic habitats yielded greater numbers of captures and species than lotic sites. Species encountered during our efforts were the same as those documented by collections from the area more than 50 years ago. This group, comprised of habitat generalists with moderately diverse aquatic resources at their disposal, appears to have done well despite dramatic anthropogenic changes to the landscape.

INTRODUCTION

State law (515 Illinois Compiled Statutes 5/1-150) directs the Illinois Department of Natural Resources to take all measures necessary for conservation of fish and aquatic life. This broad directive is refined by planning processes to identify long-term goals and actions needed to achieve them. The Illinois Comprehensive Wildlife Conservation Plan and Strategy (Illinois Department of Natural Resources 2005) embodies recent planning efforts. It advocates long-term goals of understanding the distribution and abundance of reptile and amphibian populations with confidence and conducting sentinel monitoring to identify conservation needs (Illinois Department of Natural Resources 2005: p. 41). During 2006, our agency received a State Wildlife Grant (T-10-P) to pursue these goals by documenting occurrences of reptiles and amphibians, with an emphasis on aquatic turtles. One of the areas we targeted was south-central Illinois, where records of reptiles and amphibians are considered sparse compared to many parts of the state (Wilson 1999). During 2006-2008, we sampled lentic and lotic habitats for the presence of aquatic turtles in Bond, Macoupin, and Montgomery counties to better understand their distribution, status and relative use of aquatic resources in the area.

STUDY AREA

Bond, Macoupin and Montgomery counties encompass 5,078.5 km^2 in south-central Illinois. Agriculture is the predominant land use (3,347.5 km^2; Illinois Department of Natural Resources 1996). Aquatic habitats include wetlands (84.6 km^2) and streams (31.2 km^2;

Illinois Department of Natural Resources 1996). Impoundments (47.9 km^2), mostly private ponds, are a common feature (n = 5,405) in the area (Illinois Department of Natural Resources 2001). Most large lakes (>40 ha) were created by impounding streams to provide municipal water supplies. Shoal and Macoupin creeks, the largest flowing waters in the region, are both tributaries of the Illinois River. Species documented in the study area by museum collections include snapping (*Chelydra serpentina*), painted (*Chrysemys picta*), slider (*Trachemys scripta elegans*), common musk (*Sternotherus odoratus*), and spiny softshell (*Apalone spinifera*) turtles (Phillips et al. 1999).

METHODS

We chose sampling locations (Appendix I) opportunistically based on ease and legality of access because most (>95%) property in the area is privately owned. Hoop nets, made locally by a commercial fisherman, were used to capture turtles in lentic (i.e., lakes, ponds and wetlands) and lotic habitats (i.e., small to mid-sized streams; first to fifth order) during June through August, 2006-2008. Nets were 60.96 cm in diameter and had 3.81-cm-mesh with a single "fingered" throat. We suspended a fresh or fresh-frozen bluegill (*Lepomis macrochirus*) or bass (*Micropterus salmoides*) carcass from the hoop farthest from the throat in a wire mesh container. Baits were replaced daily when we checked devices, recorded the number of each species captured and released turtles unharmed. A representative of each species was photographed at each location the first time it was encountered to serve as a record of occurrence and verify correct identification. We did not mark turtles because we anticipated that we would have too few recaptures for valid estimators of abundance.

RESULTS

We had 457 captures of turtles representing five species during 122 net-nights (2,956 hrs) of effort at 53 sites (Fig. 1). Lentic habitats yielded greater numbers of captures and species (Table 1) than lotic sites (Table 2). We detected slider, snapping, and painted turtles at most (≥ 70%) of the sites we sampled in lentic habitats. In lotic habitats, snapping and spiny softshell turtles were encountered at more sites (57% and 52%, respectively) than other species.

DISCUSSION

Comparison of our findings to those of past studies suggests we encountered all of the species that were present in our study area. It also suggests that the turtle assemblage in this part of the state is unchanged from more than 50 years ago. For example, Cahn (1937) reported four of five species (all except *S. odoratus*) from Macoupin County. Collections from Bond County in 1956 and Macoupin County during 1947 each included slider, painted, snapping, common musk and spiny softshell turtles (http://ellipse.inhs.uiuc.edu:591/INHSCollections/herpsearch; accessed 5 Sep 2008). More recently, the same five species were captured during a three-year study at a pond in Jersey County, which borders Macoupin (Reehl et al. 2006).

Readel et al. (2008) sampled 44 ponds in five counties in central and southern Illinois; they captured seven species, two of which (*Pseudemys concinna*, *Graptemys pseu-*

dogeographica) were represented by single specimens. Our study area occurs outside the range of *P. concinna* and lacks habitat suitable for *G. pseudogeographica*, which prefers large rivers and their backwaters (Phillips et al. 1999). Our lentic sites yielded greater detections of slider, painted, snapping and common musk turtles (83%, 70%, 80% and 10% of sites, respectively) than those sampled by Readel et al. (2008; 68%, 45%, 61% and 7%, respectively); the opposite was true for spiny softshells (13% vs. 18%). Given differences in geographic locations, habitats and sampling protocols, we find consistency between these studies (i.e., ranks for detections of species across sites) more remarkable than disparities in actual percentages.

Our sampling protocol was better suited for detecting the presence of species at large spatial scales than at individual sampling locations because we expended a small amount of effort (often 2-4 net-nights) per site. Nevertheless, the maximum number of species we detected at individual sites in lentic habitats ($n = 4$) is best described as "typical" for the region. Dreslik and Phillips (2005) compiled attributes of 19 chelonian communities in the upper Midwest. Species richness varied from 3-10 species, with the simplest assemblages occurring in lacustrine habitats (average species richness = 3.8) and the most diverse in ecotonal areas such as oxbows and sloughs associated with large rivers (Dreslik and Phillips 2005).

Relationships among occupancy, density and habitat correlates are poorly understood for freshwater turtles. Our data were not adequate to investigate these relationships because data were collected in an opportunistic rather than probabilistic manner. However, some of the patterns suggested by our data deserve more scrutiny using appropriate sampling designs. For example, slider and painted turtles, considered two of the most widespread and abundant species in the state, were encountered at a relatively small proportion (26%) of lotic sites. The same was true for surveys on the upper Sangamon and Mackinaw rivers, where slider and painted turtles were encountered rarely if at all (R. Bluett and C. Bartman, Illinois Department of Natural Resources, unpublished data). We speculate that *T. scripta* and *C. picta* were able to use or colonize some stream locations in Bond, Macoupin and Montgomery counties because of their proximity to reservoirs or other habitat modifications that imparted lentic characteristics to lotic sites.

Few studies have documented effects of impoundments on chelonian assemblages. Those that have (e.g., Vandewalle and Christiansen 1996, Reese and Welsh 1998) suggest negative impacts on lotic specialists (Bodie 2001). Examining effects of impoundments on assemblages comprised only of generalists, such as those found in south-central Illinois, would help to place threats from dam construction - and the need for conservation actions like mitigation - in a context that recognizes variable sensitivities of chelonian inhabitants. Efforts to control feral populations of *T. scripta elegans*, considered one of the world's most invasive species (Lowe et al. 2000), might benefit from a better understanding of whether unimpounded small to mid-sized streams serve as barriers or corridors among more favorable habitats in a landscape.

Putative threats to freshwater turtles include habitat loss, exploitation, vehicular traffic and predators (Burke et al. 2000). Types of risks and demographic responses to them vary widely among species and populations of turtles (Burke et al. 2000). As a rule, habitat specialists have fared poorly compared to generalists like slider, painted, spiny softshell

and snapping turtles (Moll and Moll 2004:9). Our findings also suggest that these generalist species remain widely distributed and common in south-central Illinois despite severe anthropogenic changes to the landscape. The status of the common musk turtle is less clear because we lack adequate historical benchmarks and knowledge about the effectiveness of baited hoop nets for their capture.

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LITERATURE CITED

Bodie, 2001. Stream and riparian management for freshwater turtles. Journal of Environmental Management 62:443-455.

Burke, V. J., J. E. Lovich, and J. W. Gibbons. 2000. Conservation of freshwater turtles. Pages 56-179 *in* M. W. Klemens, ed. Turtle Conservation. Smithsonian Institution Press, Washington, D.C., USA.

Cahn, A. R. 1937. The turtles of Illinois. Illinois Biological Monographs 16:1-2. University of Illinois, Urbana, Illinois, USA.

Dreslik, M. J., and C. A. Phillips. 2005. Turtle communities in the upper Midwest, USA. Journal of Freshwater Ecology 20:149-164.

Illinois Department of Natural Resources. 1996. Illinois land cover: an atlas. Illinois Department of Natural Resources, Springfield, Illinois, USA.

Illinois Department of Natural Resources. 2001. 2000 inventory of Illinois surface water resources. Illinois Department of Natural Resources, Springfield, Illinois, USA.

Illinois Department of Natural Resources. 2005. Illinois Comprehensive Wildlife Conservation Plan and Strategy. Illinois Department of Natural Resources, Springfield, Illinois, USA.

Lowe, S., M. Browne, S. Boudjelas and M. De Poorter. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. World Conservation Union Invasive Species Specialist Group, Gland, Switzerland.

Moll, D., and E. O. Moll. 2004. The ecology, exploitation, and conservation of river turtles. Oxford University Press, New York, New York, USA.

Phillips, C. A., R. A. Brandon and E. O. Moll. 1999. Field guide to the amphibians and reptiles of Illinois. Illinois Natural History Survey, Champaign, Illinois, USA.

Readel, A. M., C. A. Phillips and M. J. Wetzel. 2008. Leech parasitism in a turtle assemblage: effects of host and environmental characteristics. Copeia 2008:227-233.

Reehl, M., J. Thompson and J. K. Tucker. 2006. A three year survey of aquatic turtles in a riverside pond. Transactions of the Illinois State Academy of Science 99:145-152.

Reese, D. A., and H. H. Welsh, Jr.. 1998. Habitat use by western pond turtles in the Trinity River, California. Journal of Wildlife Management 62:842-853.

Vandewalle, T. J., and J. L. Christiansen. 1996. A relationship between river modification and species richness of freshwater turtles. Journal of the Iowa Academy of Science 103:1-8.

Wilson, A. K. 1999. New Illinois amphibian and reptile distribution records from the Kaskaskia River Drainage. Herpetological Review 30:118-120.

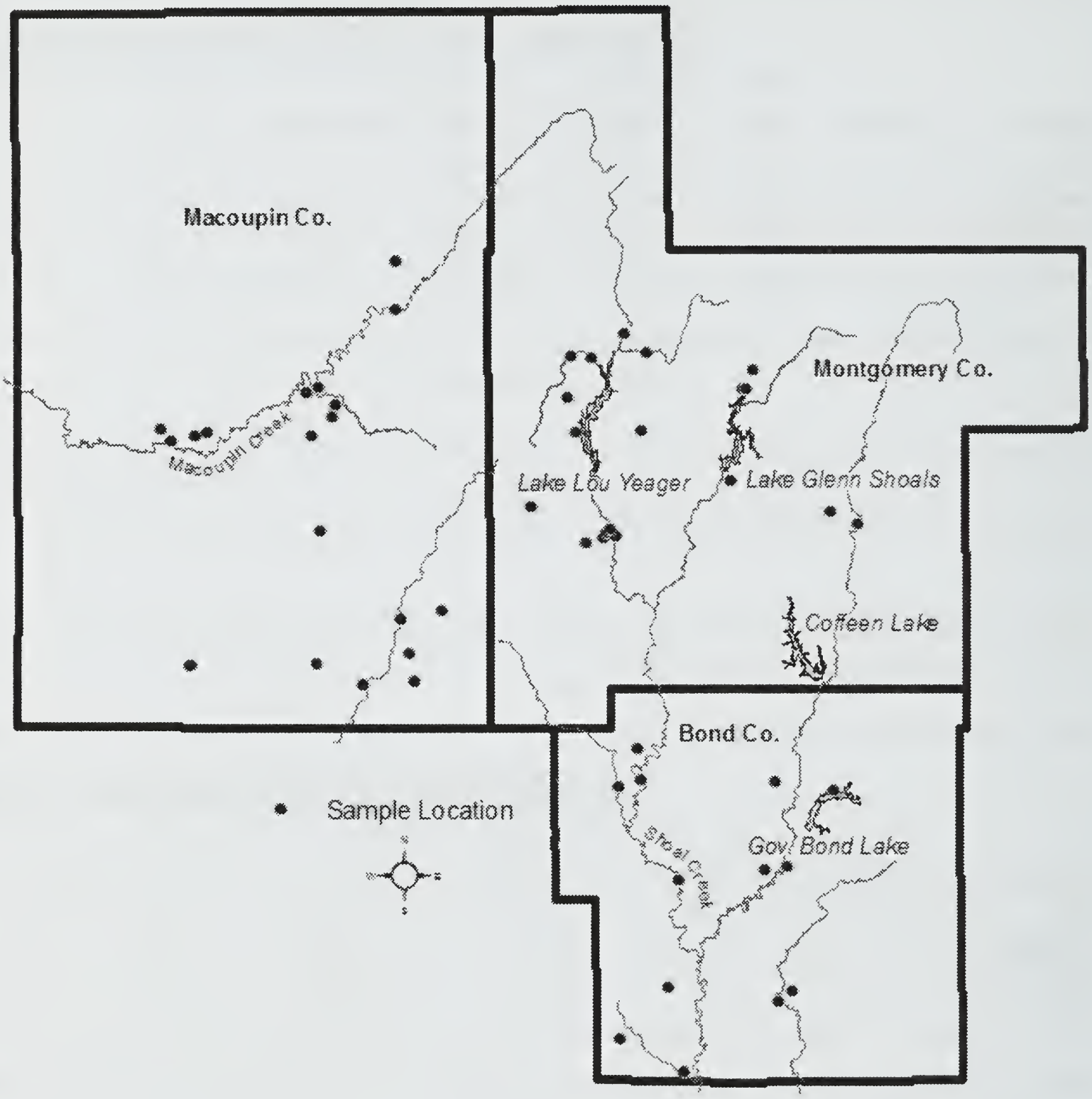
Table 1. Turtles captured in lentic habitats in Bond, Macoupin, and Montgomery counties, Illinois, 2006-2008.

			No. captures (No. sites where captured)				
County	No. Sites	Effort (hrs)	*Trachemys scripta*	*Chrysemys picta*	*Chelydra serpentina*	*Apalone spinifera*	*Sternotherus odoratus*
Bond	7	434	41 (5)	36 (5)	5 (4)	2 (1)	1 (1)
Macoupin	13	742	126 (12)	15 (7)	22 (11)	1 (1)	1 (1)
Montgomery	10	604	66 (8)	37 (9)	22 (9)	4 (2)	3 (1)
Total	30	1780	233 (25)	88 (21)	49 (24)	7 (4)	5 (3)

Table 2. Turtles captured in lotic habitats in Bond, Macoupin, and Montgomery counties, Illinois, 2006-2008.

			No. captures (No. sites where captured)			
County	No. Sites	Effort (hrs)	*Trachemys scripta*	*Chrysemys picta*	*Chelydra serpentina*	*Apalone spinifera*
Bond	6	290	0 (0)	1 (1)	3 (3)	9 (3)
Macoupin	7	369	4 (3)	0 (0)	5 (3)	9 (5)
Montgomery	10	519	10 (3)	15 (5)	8 (7)	11 (4)
Total	23	1176	14 (6)	16 (6)	16 (13)	29 (12)

Figure 1. Sites sampled for the presence of aquatic turtles in Bond, Macoupin, and Montgomery counties, Illinois, 2006-2008.



APPENDIX 1.

Sampling locations, habitat types and captures of freshwater aquatic turtles in Bond, Macoupin and Montgomery counties, Illinois, 2006-2008.

			No. captures				
County	Habitat	Latitude/longitude	*Trachemys scripta*	*Chrysemys picta*	*Chelydra serpentina*	*Apalone spinifera*	*Sternotherus odoratus*
Bond	pond	39.9597/-89.4306	0	1	0	0	0
Bond	pond	38.8094/-89.5307	8	1	1	0	0
Bond	lake	38.9538/-89.3743	0	3	0	2	1
Bond	lake	38.8957/-89.4389	8	0	1	0	0
Bond	lake	38.8065/-89.4133	5	0	2	0	0
Bond	lake	38.7718/-89.5748	6	1	1	0	0
Bond	lake	38.9841/-89.5593	14	30	0	0	0
Macoupin	wetland	39.2118/-89.9779	0	2	1	0	0
Macoupin	wetland	39.2353/-89.8454	5	2	0	0	0
Macoupin	lake	39.2470/-89.8623	4	4	1	0	1
Macoupin	lake	39.1426/-89.8593	2	0	1	0	0
Macoupin	pond	39.0436/-89.9809	9	3	1	0	0
Macoupin	lake	39.0844/-89.7441	1	0	1	0	0
Macoupin	pond	39.0457/-89.8629	4	1	1	0	0
Macoupin	lake	39.2162/-90.0116	65	0	5	1	0
Macoupin	pond	39.2266/-89.8480	7	2	1	0	0
Macoupin	lake	39.3392/-89.7886	11	1	4	0	0
Macoupin	lake	39.2124/-89.8675	11	0	1	0	0
Macoupin	lake	39.0527/-89.7754	3	0	0	0	0
Macoupin	lake	39.0323/-89.7700	4	0	5	0	0
Montgomery	lake	39.2153/-89.6182	2	2	2	1	0
Montgomery	lake	39.1800/-89.4715	17	4	3	0	3
Montgomery	pond	39.2159/-89.5571	0	1	4	0	0
Montgomery	pond	39.2465/-89.4600	4	6	1	0	0
Montgomery	pond	39.1567/-89.3786	0	0	1	0	0
Montgomery	pond	39.1377/-89.5921	6	2	0	0	0
Montgomery	pond	39.1416/-89.5880	5	10	2	0	0
Montgomery	wetland	39.1387/-89.5808	5	3	1	0	0
Montgomery	pond	39.2409/-89.6259	11	8	4	0	0

			No. captures				
County	Habitat	Latitude/longitude	*Trachemys scripta*	*Chrysemys picta*	*Chelydra serpentina*	*Apalone spinifera*	*Sternotherus odoratus*
Montgomery	lake	39.1606/-89.6600	16	1	4	3	0
Bond	stream	38.7991/-89.4262	0	1	0	0	0
Bond	stream	38.9562/-89.5765	0	0	0	0	0
Bond	stream	38.7483/-89.5155	0	0	1	0	0
Bond	stream	38.8875/-89.5208	0	0	0	2	0
Bond	stream	38.9610/-89.5569	0	0	1	6	0
Bond	stream	38.8984/-89.4195	0	0	1	1	0
Macoupin	stream	39.0307/-89.8179	1	0	0	0	0
Macoupin	stream	39.0789/-89.7826	0	0	1	2	0
Macoupin	stream	39.2440/-89.8733	1	0	0	1	0
Macoupin	stream	39.2143/-89.9672	2	0	3	3	0
Macoupin	stream	39.3042/-89.7886	0	0	1	0	0
Macoupin	stream	39.2078/-90.0014	0	0	0	2	0
Macoupin	stream	39.0429/-89.9827	0	0	0	1	0
Montgomery	stream	39.2701/-89.6232	0	0	1	0	0
Montgomery	stream	39.2696/-89.6040	4	1	1	0	0
Montgomery	stream	39.2730/-89.5518	0	0	1	0	0
Montgomery	stream	39.1345/-89.6088	0	0	1	0	0
Montgomery	stream	39.2466/-89.4575	1	2	0	1	0
Montgomery	stream	39.2612/-89.4516	5	8	0	1	0
Montgomery	stream	39.1387/-89.5808	0	3	1	3	0
Montgomery	stream	39.1446/-89.5848	0	0	1	6	0
Montgomery	stream	39.1486/-89.3522	0	0	0	0	0
Montgomery	stream	39.2868/-89.5735	0	1	2	0	0

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Habituation and Dishabituation to a Vibrational Stimulus by a Millipede, *Orthoporus texicolens*

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ABSTRACT

The primary defense mechanism of the North American millipede, *Orthoporus texicolens*, is coiling. We studied habituation, a form of non-associative learning, by observing coiling in response to a vibrational stimulus. Coiling declined in successive trials, indicating that habituation occurred. Dishabituation was then studied by not presenting the vibrational stimulus for intervals ranging from 4-14 minutes. The response of coiling did begin to reappear, showing evidence of dishabituation. Of the experimental millipedes, 75% habituated to vibrational stimuli, and of these millipedes, 100% dishabituated due to the lengthening time span between each vibrational period. Our data indicate that millipedes are able to habituate to a stimulus and then dishabituate to the stimulus if the time intervals between stimulus presentation are lengthened.

INTRODUCTION

Habituation is a type of non-associative learning when an organism learns not to respond to an irrelevant stimulus that initially elicited a response (Muller and Hildebrandt, 2002). Habituation is important for organisms receiving multiple sensory cues from their environment because it allows for stimulus filtering (Gray, 2005), thus enabling organisms to learn to react only to biologically relevant environmental stimuli (Mankin et al., 1999; Best et al., 2005). For example, avoiding predation becomes more likely by focusing on stimuli produced by predators; less threatening stimuli do not trigger a response, thus reducing the amount of time expended on irrelevant stimuli (Gray, 2005). In order for habituation to occur, the organism must not be food deprived or unhealthy (Braun and Bicker, 1992), and the stimulus must be presented repeatedly (Wyttenbach and Hoy, 1997). Often habituation is considered to be short term (Engel and Hoy, 1999) and is considered a simple form of learning (Bristol and Carew, 2005).

Various invertebrates such as honeybees (Braun and Bicker, 1992), crickets (Engle and Hoy 1999), leeches (Burrell et al., 2001), flatworms (Koopowitz, 1974), and mollusks (Bristol and Carew, 2005) have been shown to habituate to irrelevant stimuli. Once a habituated organism comes in contact with a different form of the stimulus, it may respond as if the stimulus is novel. This new response is known as dishabituation (Burrell et al., 2001). It is not adaptive for habituation to occur without the mechanism for dishabituation (Krasne and Glanzman, 1995).

Habituation in millipedes has not previously been studied. The purpose of the study was to examine whether millipedes habituate to a vibrational stimulus and then to test for dishabituation.

METHODS

The organisms tested were North American millipedes, *Orthoporus texicolens*. Each millipede was housed separately in a holding facility (11.0 cm diameter x 10.0 cm h) containing moist topsoil, and food and water were provided *Ad libitum*.

Millipedes avoid predators by using two different defense mechanisms - excreting a noxious substance or coiling into a ball with the head tightly covered. Since not all species use chemical secretions to deter predators (Arab et al., 2003), we used millipede coiling to indicate defensive behavior. We used the smallest possible frequency of vibration to illicit a response from the millipedes since weaker stimuli require decreased time for habituation to occur compared to stronger stimuli (Koopowitz, 1974).

We used 40 millipedes randomly separated into 20 control millipedes and 20 experimental millipedes. The experimental millipedes received the vibrational stimuli repeatedly to test for habituation, while the control millipedes only received two vibrational stimuli separated by a 78 minute time interval.

Each millipede was positioned on top of a platform that was directly in contact with a vibrator (an Elenco GF- 8026 function generator hooked to Pasco Scientific SF- 9324 mechanical vibrator). In order to examine habituation, experimental millipedes were exposed to a vibrational stimulus of 37 hertz for 5 seconds. We conducted 40 trials on each millipede with 2 minute intervals between each vibration. Control millipedes were only exposed to the vibrational stimulus twice for 5 seconds, with the stimuli separated by a 78 minute time interval. Presence or absence of millipede coiling was recorded for all trials.

We then exposed all experimental and control millipedes to the same conditions to examine possible dishabituation responses in each of the two groups. We exposed millipedes to additional vibrations after trial number 40, but instead of a 2 minute interval between vibrations, one stimulus was skipped, leaving a 4 minutes interval until the next stimulus. Next, two stimuli were skipped leaving a 6 minute interval. This pattern of skipping stimulation continued until 6 vibration periods were skipped, leaving an interval of 14 minutes between stimuli. Similar methodology of altering interval lengths between the previously used stimulus was employed to test for dishabituation of acoustic startle stimuli in mice (Sasaki et al., 2001)

We used two regression analyses to determine the change in proportion of millipedes that responded over time. One analysis represents presence of the coiling response of millipedes over time and the other represents absence of the coiling response in millipedes over time.

RESULTS

Since the research consisted of two different experiments (habituation and dishabituation), two sets of data were collected. At the beginning of the habituation experiment, 95% of the experimental millipedes coiled; whereas, after 40 trials, significantly less (25%) continued to coil (P = 2.15E-22) (Fig. 1). In contrast, 95% of the control millipedes initially coiled, and after our interval of 78 minutes, 90% still responded (Fig. 1).

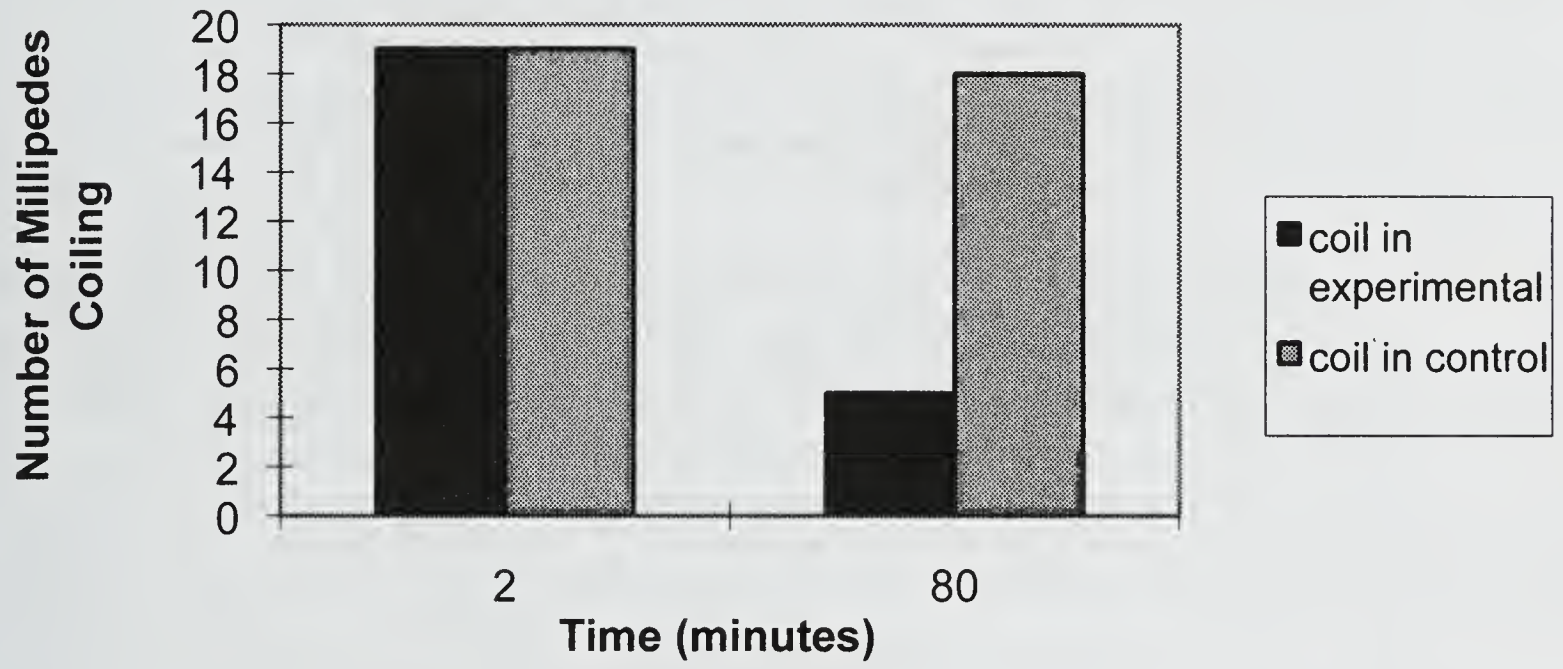

Figure 1. Habituation study: Response of experimental millipedes (subjected to 40 vibrations at 2 minute intervals) and control millipedes (subjected to only 2 vibrations with an 78 minute interval) during the first and last trials.

The dishabituation experiment involved 15 habituated experimental millipedes that no longer responded to the vibrations and 15 control millipedes. During the first trial of the dishabituation research, none of the experimental millipedes responded to the vibrational stimulus (Fig. 2). However, significantly more (100%) of the experimental millipedes responded during the last trial conducted 54 minutes later (P = 0.000439) (Fig. 2). In contrast, 93% of the control millipedes responded to the first trial vibrational stimulus, and 100% still responded after the 54-minute interval (P = 0.140) (Fig. 2).

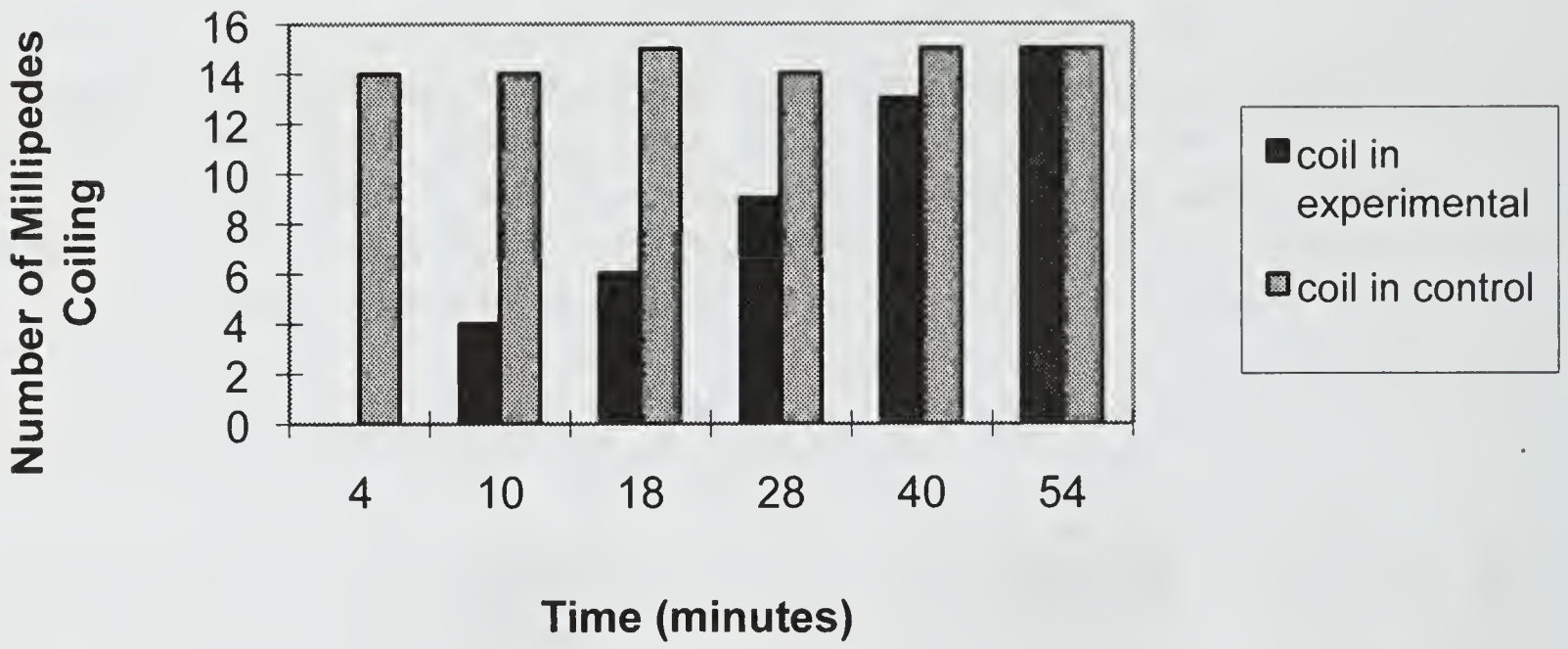

Figure 2. Dishabituation study: 15 habituated experimental and 15 control millipedes' reactions to the vibrational stimulus. Each time interval between the vibration stimuli was 2 minutes longer than the previous vibration causing longer time periods between vibrations to occur in successive trials.

DISCUSSION

Experimental millipedes exposed to repeated vibrations did habituate while control millipedes, exposed to only two vibrations, did not. Initially, the response to the vibrational stimulus was coiling, but toward the end of the trials, experimental millipedes no longer responded while control millipedes continued to respond. Habituation occurs in response to a repeated irrelevant stimulus (May and Hoy, 1991). Millipedes initially responded via coiling because the stimulus was novel. After repeated trials, millipedes learned not to respond to the irrelevant stimulus. Thus, non-associative learning was demonstrated for the first time in a species of millipede. There is additional support for habituation in uniramia since, upon exposure to a repeated electrode stimulus, some insects also habituated (Braun and Bicker, 1992; Engle and Hoy, 1999; Friedel, 1999). For example, locusts undergo habituation by decreasing startle responses to repeated vibrational stimuli that are not associated with reward or punishment (Friedel, 1999).

Dishabituation also occurred as indicated by the experimental millipedes response to the vibration as time between trials increased. The longer the time span between vibrational stimuli, the more novel the stimulus seemed; therefore, habituation was no longer intact (Wyttenbach and Hoy, 1997). Since the control millipedes had not undergone habituation, their response rate remained constant throughout.

Our research demonstrates the presence of non-associative learning (habituation) and subsequent dishabituation in an invertebrate. This is the first study of habituation and dishabituation in millipedes. Further research should explore the role of associative learning in these invertebrates.

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LITERATURE CITED

Arab, A., C.G. Zacarin, C.S. Fontanetti, M.I. Camargo-Mathias, M.G. dos Santos, and A.C. Cabrera. 2003. Composition of the defensive secretion of the Neotropical millipede *Rhoncricus padbergi* Verhoeff 1938 (Diplopoda: Spirobolida: Rhinocricidae). Entomotropica. 18: 79-82.

Best, A.R and J.V. Thompson, M.L. Fletcher, and D.A. Wilson. 2005. Cortical metabotropic glutamate receptors contribute to habituation of a simple odor-evoked behavior. The Journal of Neuroscience. 25(10): 2513-2517.

Braun, G. and G. Bicker. 1992. Habituation of an appetitive reflex in the honeybee. Journal of Neurophysiology. 67:588-598.

Bristol, A.S. and T.J. Carew. 2005. Differential role of inhibition in habituation of two independent afferent pathways to a common motor output. Learning and Memory. 12: 52-60.

Burrell, B.D., C.L. Sahley, and K.J. Muller. 2001. Non-Associative learning and serotonin induce similar bi-directional changes in excitability of a neuron critical for learning in the medicinal leech. The Journal of Neuroscience. 21:1401-1412.

Engel, J.E. and R.R. Hoy. 1999. Experience-dependent modification of ultrasound auditory processing in a cricket escape response. The Journal of Experimental Biology. 202: 2797-2806.

Friedel, T. 1999. The vibrational startle response of the desert locust *Schistocerca gregaria*. The Journal of Experimental Biology. 202: 2151-2159.

Gray, J.R. 2005. Habituated visual neurons in locusts remain sensitive to novel looming objects. The Journal of Experimental Biology. 208: 2515-2532.

Koopowitz, H. 1974. Activity and habituation in the brain of the polyclad flatworm *Freemania litoricola*. The Journal of Experimental Biology. 62: 455-467.

Krasne, F.B. and D.L. Glanzman. 1995. What we can learn from invertebrate learning. Annual Review of Psychology. 46:585-624.

Mankin, R.W., R.T. Arbogast, P.E. Kendra, and D.K. Weaver. 1999. Active spaces of pheromone traps for *Plodia interpunctella (Lepidoptera: Pyralidae)* in enclosed environments. Environmental Entomology. 28: 557-565.

May, M.L. and R.R. Hoy. 1991. Habituation of the ultrasound-induced Acoustic startle response in flying crickets. Journal of Experimental Biology. 159: 489-499.

Muller, U. and H. Hildebrandt. 2002. Niric oxide/cGMP-medicated protein kinase A activation in the antennal lobes plays an important role in appetitive reflex habituation in the honeybee. The Journal of Neuroscience. 22: 8739-8747.

Sasaki, A., W. C. Wetsel, R. M. Rodriguiz, and W. H. Meck. 2001. Timing of the acoustical startle response in mice: Habituation and dishabituation as a function of the interstimulus interval. International Journal of Comparative Psychology. 14:258-268.

Wyttenbach, R.A. and R.R. Hoy. 1997. Spatial acuity of ultrasound hearing in flying crickets. Journal of Experimental Biology. 200: 1999-2006.

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A Nuclear Gene Investigation of the Sunfish Genus *Lepomis* (Perciformes: Centrarchidae)

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ABSTRACT

In this paper, we test the phylogenetic utility of the nuclear gene beta-actin for the sunfish genus *Lepomis* using maximum parsimony and maximum likelihood analyses. Using only the beta-actin sequence all major species-clades were recovered in both analyses. However, more basal nodes were only weakly supported. Results indicate that beta-actin performs moderately well as a phylogenetic marker and should be subjected to further use. Secondly, we explore the utility of the beta-actin gene to develop a rapid, non-lethal polymerase chain reaction (PCR)-based species identification method. We developed taxon-specific primers to identify *Lepomis* species based on polymorphisms in the introns of the beta-actin nuclear gene. Products were diagnostic for all species except for the *L. cyanellus/L. symmetricus* and *L. minatus/L. punctatus* species pairs.

INTRODUCTION

The sunfish genus *Lepomis* is comprised of 12 species endemic to eastern North America: *Lepomis auritus* (redbreast sunfish); *L. gibbosus* (pumpkinseed); *L. gulosus* (warmouth); *L. microlophus* (redear sunfish); *L. cyanellus* (green sunfish); *L. macrochirus* (bluegill); *L. marginatus* (dollar sunfish); *L. megalotis* (longear sunfish); *L. miniatus* (redspotted sunfish); *L. punctatus*(spotted sunfish); *L. symmetricus*(bantam sunfish); *L. humilis* (orangespotted sunfish); (Page and Burr, 1991). The genus is about 15 million years old (Bolnick et al., 2006) and given its wide distribution and abundance has been subjected to a series of evolutionary investigations using several markers, including: allozymes, external morphology, osteology, mitochondrial DNA, and nuclear DNA (Wainwright and Lauder, 1992; Mabee, 1993; Near et al., 2004; Harris et al., 2005; Bolnick et al., 2006). Researchers have consistently recovered the more basal patterns of evolutionary divergence in *Lepomis* including the early divergence of a clade containing *L. cyanellus* and the recognition of some major species complexes such as *L. megalotis – L. marginatus* (Near et al., 2004; Harris et al., 2005; Bolnick et al. 2006). However, species-level relationships remain largely unresolved and certain species, such as *L. auritus* and *L. gibbosus*, repeatedly fall out in different places within phylogenies. It is unclear

why hypotheses of *Lepomis* relationships show such inconsistencies but possible reasons include rapid historical diversification, inadequate analyses of phylogenetic data, inadequate markers or taxon sampling, genetic introgression via historical and recent hybridization, or cryptic diversity (Near et al., 2004; Harris et al., 2005).

The objectives of this study are two-fold. First, we investigate the phylogenetic utility of the nuclear gene beta-actin using the sunfish genus *Lepomis* as the exemplar. We analyze the performance of the marker by estimating phylogenies using maximum parsimony and maximum likelihood tree estimation techniques. Our results are compared to published phylogenetic hypotheses of the genus.

Secondly, we explore the use of the EPIC-PCR approach to derive species-specific molecular markers for members of genus *Lepomis* using beta-actin. Given the frequency of misidentifications due to morphological similarities (e.g., juveniles often require scale counts to correctly identify species) and phenotypic plasticity (Harris et al., 2005), we evaluate the utility of our nuclear DNA primers for performing rapid PCR-based techniques that exploit variation in intron sequences for species identification. The importance of developing a technique for identifying fishes via a rapid, non-lethal genetic screening can be dramatic for some species, especially if the fish in question is endangered, threatened, or locally rare.

METHODS

Tissues samples were taken from individuals collected from different locations within their respective ranges. The *L. cyanellus* × *L. microlophus* hybrid specimens were artificially produced. In a previous study, we examined the entire beta-actin gene and found that the majority of the intronic differences between *L. cyanellus* and *L. macrochirus* occurred in the first two (of four) sequenced introns (Peyton, 2004). Based on that observation, we focused this study on sequencing the first 1000 nucleotides from the remaining ten species. This genomic fragment contains all but 27 nucleotides of exon 1, all of intron 1, all of exon 2, and most of intron 2.

Phylogenetic Analyses of Beta-actin Gene Sequences

The beta-actin sequences were aligned via a combination of manual and automatic techniques using BioEdit (Hall, 1999) and the Vector NTI software suite. Phylogenies were estimated using maximum parsimony (MP) and maximum likelihood analyses (ML) using PAUP* (D.L. Swofford, Sinauer Associates, INC., Sunderland, MA, 2002). Optimal trees were found via a heuristics search of 1000 pseudoreplicates with tree bisection-reconnection. A bootstrap analysis consisting of 1000 pseudoreplicates and 100 random addition sequences was completed for each analysis. Branches with bootstrap support of less than 50% were collapsed.

Modeltest (Posada and Crandall, 1998) was used to identify the appropriate parameters for the ML analysis. The ML parameters were as follows: A = 0.2772, C = 0.2350, G = 0.1956, T = 0.2922, A-C = 0.9629, A-G = 1.8763, A-T = 0.9282, C-G = 0.4553, C-T = 1.8763, G-T = 1.000, Ti/Tv = 1.0746, proportion of invariable sites = 0 and discrete gamma distribution shape parameter = 0.8325. For the MP analysis, gaps were treated as a fifth character.

Species-specific Oligonucleotide Primers

DNA was isolated from fin clip biopsies using the Qiagen DNeasy Kit. Three microliters were used for subsequent PCR reactions. Beta-actin gene primers were designed to hybridize with the first 9 codons and a conserved region in the second intron in accordance with EPIC-PCR techniques. Primer sequences were as follows: Forward: 5' atg gat gat gaa atc gcc gca ctg gtt 3'; Reverse: 5' aga taa ggc aca cag tca aga gag acc tgt 3'. Cycle parameters were: 95° C for 2 minutes, followed by 35 cycles of (95° C for 30sec, 47° C for 30sec, 72° C for 1minute). The genomic fragment produced from each fish was approximately 1 kb and was cloned into the pGEM-T Easy vector (Promega). Candidate clones were sequenced by MWG Biotech, Inc. and compared to known beta-actin genes for verification, and the exons were determined using consensus splice sites and the virtually invariant beta-actin amino acid sequence. The sequence data for *L. cyanellus* and *L. macrochirus* were taken from a previous study (Peyton 2004).

The polymerase chain reaction was carried out with each sample using the following amounts: 3 μl genomic DNA , 1.5 μl of each primer (from a 10 ng/μl stock), 12.5 μl of 2× GoTaq PCR master mix (Promega), and water up to 25 μl. Primer sequences, annealing temperature used, and number of cycles are given in Table 1. Samples were electrophoresed on a 1.8% agarose gel and visualized using ethidium bromide staining.

RESULTS

Phylogenetic Analyses

In total there were 280 parsimony informative characters. The monophyly of *Lepomis* was well supported in each analysis and is in agreement with recent phylogenetic studies of the Centrarchidae genera (Near et al., 2004; Harris et al. 2005). The MP analysis provided a moderate level of resolution (Figure 1). Notwithstanding the placement of *L. gulosus*, *L. gibbosus*, and *L. auritus*, all traditionally recognized species complexes within *Lepomis* were resolved with the MP analysis. The ML tree (Figure 2) was slightly less resolved than the MP tree. Again, traditional species complexes were recovered but the more basal relationships were poorly supported and the major species groups were collapsed into a polytomy based on weak nodal support.

Species-Specific Primers and PCR-based Identification

The gel images in Figure 3 illustrate typical screening results for each primer set detailed in Table 1. Clear identification was possible for each species except for distinction between *L. cyanellus/L. symmetricus* and *L. miniatus/L. punctatus*, respectively. In the case of the former pair, *L. cyanellus* and *L. symmetricus*, we had clear and reproducible bands for both using multiple primer pairs, but none could robustly distinguish the subtle nucleotide differences between the two. This explains the result that the hybrid (*L. cyanellus* cross to *L. microlophus*) is identified as *L. microlophus, L. cyanellus,* and *L. symmetricus*. In the case of the latter pair, *L. miniatus* and *L. punctatus*, we found that one primer pair effectively identifies *L. miniatus*, but the corresponding pair is only marginally effective at identifying *L. punctatus*. Attempts to use alternate primer pairs, or to reduce the stringency of the reaction, resulted in products for both species.

DISCUSSION

Phylogenetic Analyses

The MP tree was similar to nearly all published hypotheses of species relationships and recovered essentially all major species groups despite being constructed using only a single nuclear gene (Avise et al., 1977; Wainright and Lauder, 1992; Mabee, 1993; Near et al., 2004; Harris et al., 2005). The position of *L. gibbosus* was most enigmatic and was placed basal to all other *Lepomis* species. Other hypotheses (Near et al., 2004; Harris et al., 2005) place *L. gibbosus* in a more terminal position and sister to a clade consisting of *L. microlophus*, *L. minatus*, and *L. punctatus*. The position of *L. auritus* was also different from other hypotheses, however, Harris et al. (2005) noted that the recovery of a consistent placement of *L. auritus* has not been achieved and attributed much of this due to historical genetic introgression with other *Lepomis*. The MP analysis also resolved *L. gulosus* as sister to a clade containing *L. cyanellus*, *L. symmetricus*, *L. macrochirus*, and *L. humilis*. Other hypotheses (Near et al., 2004; Harris et al., 2005) consistently place *L. gulosus* as embedded within this clade and sister to the complex including *L. cyanellus* and *L. symmetricus*. In contrast, the position of *L. gulosus* was different in the ML analysis and that species was shown sister to the *L. cyanellus* – *L. symmetricus* clade which is in general agreement with other hypotheses. The position of *L. gibbosus* remained uncertain and these specimens comprised one of the four collapsed branches on the tree. *Lepomis auritus* was still shown as belonging to the *L. punctatus* – *L. minatus* clade.

In summary, the recovery of major species complexes and moderate resolution of more basal relationships within the genus *Lepomis* suggests that beta-actin is a potentially useful phylogenetic marker. Because we were primarily focused on generally testing the phylogenetic utility of beta-actin, our sample size is inadequate for developing a robust hypothesis of relationships with *Lepomis*. Additional sampling is needed to test for intraspecific variation not accounted for in the current study. The results, however, are encouraging and this marker should be subjected to further scrutiny using more comprehensive sampling and perhaps combining the data with that of previously published hypotheses.

Species-specific Oligonucleotide Primers

In the *Lepomis* specimens we examined we found ample polymorphic sites to distinguish the majority of the twelve species. Even among the species that were not reliably distinguished, *L. cyanellus/L. symmetricus* and *L. miniatus/L. punctatus*, there were sufficient SNPs to distinguish the species by sequence. It was our design to not rely on SNPs because of the probability that these substitutions could occur by chance in another species, eliminating the position as a species-specific character. All of the primers utilized in this study were designed to take advantage of multiple mismatches occurring in succession, or when possible to anneal to an insertion present in only a single case. Polymorphisms involving multiple nucleotide mutations are most likely the result of several mutational events, occurring over the course of more than one generation. An important consideration about the efficacy of this molecular test is whether the differences observed in the *Lepomis* sequences are stable across populations. In this study we used a limited number of specimens and further research is needed with a geographically expanded sample size to rigorously test for intraspecific stability. However, in a previous study our

primers recognized *L. macrochirus* taken from disparate geographical locations in the eastern US (Peyton, 2004).

The *L. miniatus/L. punctatus* complex provides an interesting example of how similar two sequences can be for separate species. *Lepomis miniatus*, was only recently elevated to species status, having previously been a subspecies of the spotted sunfish, *L. punctatus* (Warren, 1992). Their phylogenetic closeness is evident in each of our phylogenetic analyses: a close analysis of the alignment between the two sequences shows that the divergence consists of SNPs spaced out over the length of the two fragments. This is what we would expect as the result of random genetic drift occurring over an evolutionarily short period of time, as compared to the gaps and insertions seen among the other species, which would take longer as mentioned. A 1.7% difference in sequence composition is sufficient, hypothetically, to design allele-specific primers but we found the results of our allele-specific primers for these species to be less than satisfactory.

The use of molecular tests for species identification is becoming more widespread (Hebert at al., 2004; Noël et al., 2008). In the case of DNA barcoding (Herbert et al., 2004), a standard genomic position is sequenced and compared to a database. Each individual specimen must be sequenced for this region and potential problems arise when examining specimens of hybrid origin or taxa with extensive historical genetic introgression. Noël et al. (2008) used a technique similar to the one used here to design specific primers for identifying morphologically ambiguous individuals from unisexual populations of rare *Ambyostoma* salamanders. In their study, the primers they designed did not discriminate between species and only produced a positive/negative result for unisexual population origin.

The development of species or strain-specific tests for fishes has lagged due to continued use of allozyme tests and microsatellites. The former requires lethal sampling of tissues and organs, the latter is primarily suited for intraspecific population studies (DeWoody et al., 2000). Because microsatellites exhibit a degree of hypervariability, that likely makes them unsuitable for species identification. A solution to this problem is to find a gene that can be easily amplified and sequenced from any species, and to use polymorphisms within that gene which are likely to be conserved across populations.

The beta-actin gene is a good candidate for species identification because it is highly conserved at the amino acid level, suggesting that universal primers will be functional across multiple taxa (Baldauf et al., 2000). Our experience has indicated that the same beta-actin primers used in this study also can amplify beta-actin genes from species within Esocidae and Cyprinidae. However, potential complications arise when dealing with closely related species (e.g., *L. minatus/L. puntatus* and *L. cyanellus/L. symmetricus*).

Moreover, fishes present problems with contemporary hybridization and historical genetic introgression common in many groups (Hopkins and Eisenhour, 2008). However, in most natural *Lepomis* populations, there are perhaps relatively few hybrids between recognized species that are not identifiable as the F_1 generation (Avise and Saunders, 1984). As demonstrated, the primers described in this paper can be used to identify F_1 hybrids. More extensive hybridization cannot be distinguished with our primers and would certainly confound the screening results. In fact, hybridization and introgression

that extends beyond the first generation would confound even multi-gene screening tests. Determination of parentage for deeply introgressed specimens would approach speculation considering the randomizing powers of meiotic cross-over events, independent assortment of chromosomes, and the potential for instraspecific variation from individual to individual. A larger survey of multiple populations of sunfish would be necessary to determine if our primers can rigorously work on sunfish from distinct geographical regions.

In summary, we have utilized the intronic polymorphisms of the highly conserved beta-actin gene to develop oligonucleotide primers that can distinguish among most species of *Lepomis* sunfish and can be used to verify the parental species of a putative F_1 hybrid via a rapid PCR-based screening. The conserved nature of the beta-actin sequence makes this test amenable to development for other genera as well, and may have applications for identifying fish species from degraded samples (e.g., stomach contents), species with uninformative morphological features, matching larval fishes to adult species, or non-lethal identification of rare species.

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MATERIALS EXAMINED

Specimens were collected in the following locations: *Lepomis auritus* (MOSU 2340), Bell County, KY; *L. gibbosus* (MOSU 2333), Horry County, SC; *L. gulosus* (MOSU 2347) and *L. microlophus* (MOSU 2343), Clear Creek Lake in Bath County, KY; *L. cyanellus* (MOSU 2344) and *L. macrochirus* (MOSU 2345), South Elkhorn Creek in Woodford County, KY; *L. macrochirus* (SIUC 37963), Illinois; *L. marginatus* (MOSU2346), Graves County, KY; *L. megalotis* (MOSU 2348), Rowan County, KY and (UF146998), Monroe County, Georgia; *L. miniatus* (MOSU 2342), Mud River in Logan County, KY; *L. punctatus* (MOSU 2339), Horry County, SC; *L. symmetricus* (MOSU 2341), Graves County, KY; *L. humilis* (University of Tennessee Tissue Collection INHS 42594, SIUC 37962(2), IL; *M. dolomieu* (MOSU 2349), Rowan County, KY; *M. punctulatus* (MOSU 2350), Rowan County, KY; *L. cyanellus* × *L. microlophus* hybrid specimens were artificially produced as described.

REFERENCES

Avise JC, Straney DO, Smith MH. 1977. Biochemical genetics of sunfish IV. Relationships of centrarchid genera. Copeia 2:250-258.

Avise JC, Saunders NC. 1984. Hybridization and Introgression Among Species of Sunfish (*Lepomis*): Analysis by Mitochondrial DNA and Allozyme Markers. Genetics 108:237- 255.

Baldauf SL, Roger AJ, Wenk-Siefert I, Doolittle WF. 2000. A Kingdom-Level Phylogeny of Eukaryotes Based on Combined Protein Data. Science 290:972-977.

Bolnick DI, Near TK, Wainwright PC. 2006. Body size divergence promotes post-zygotic reproductive isolation in centrarchids. Evolutionary Ecology Research 8:903-913.

DeWoody JA, Fletcher DE, Wilkins SD, and Avise JC. 2000. Parentage and nest guarding in the Tessellated Darter (*Etheostoma olmstedi*) assayed by microsatellite markers. Copeia 3:740-747.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

Harris PM, Roe KJ, Mayde RL. 2005. A mitochondrial DNA perspective on the molecular systematics of the sunfish genus *Lepomis* (Actinopterygii: Centrachidae). Copeia 2:340- 346.

Hebert, PDN, Penton EH, Burns JM, Jansen, DH, and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceeding of the National Academy of Sciences 101(4):14812-14817.

Hopkins RL, Eisenhour DJ. 2008. Hybridization of *Lythrurus fasciolaris* (Cope) and *Lythrurus umbratilis* (Girard) (Cypriniformes: Cyprinidae) in the Ohio River basin. Copeia 1:162- 171.

Mabee PM. 1993. Phylogenetic interpretation of ontogenetic change: sorting out the actual and artifactual in an empirical case study of centrarchid fishes. Zool. J. Linnearn Soc., 107:175-291.

Near TJ, Bolnick DI, Wainright PC. 2004. Investigating phylogenetic relationships of sunfishes and black basses (Actinopterygii: Centrarchidae) using DNA sequences from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution 32:344-357.

Noël S, Dumoulin J, Ouellet, M, Galois P, Lapointe FJ. 2008. Rapid identification of salamanders from the Jefferson complex with taxon-specific primers. Copeia 1:158-161.

Page, LM and Burr, BM. 1991. A Field Guide to Freshwater Fishes. Houghton Mifflin Company. New York, NY

Peyton DK. 2004. A PCR-Based F1 Hybrid Screen Using the Beta-actin Genes from the Sunfishes *Lepomis cyanellus* and *L. macrochirus* (Centrarchidae). The Journal of the Kentucky Academy of Sciences 65:104-107.

Posada D, Crandall KA. 1998. Model Test: testing the model of DNA substitution. Bioinformatics 14:817-818.

Swofford DL, Olson GJ, Waddell, Hillis DM. 1996. Phylogenetic inference, p. 407-517. *In*: Molecular Systematics. DM Hillis, C Moritz, and BK Mable (eds). Sinauer Associates, Sunderland, Massachusetts.

Wainright PC, Lauder GV. 1992. The evolution of feeding biology in sunfishes (Centrarchidae). In: Mayden RL (Ed), Systematics, Historical Ecology, and North American Freshwater Fishes. Standford University Press. Stanford, CA. pp. 472-491.

Warren, ML. 1992. Variation of the Spotted Sunfish, *Lepomis punctatus* Complex (Centrarchidae): Meristics, Morphometrics, Pigmentation and Species Limits. Alabama Museum of Natural History Bulletin 12.

Table 1. Sequences, annealing temperature, and cycle number for species-specific oligonucleotide primers.

Species	Primers	Annealing Temp	Cycles
L. auritus	*Laur 113F cca ggc atc agg tga gca caa* *Laur 270R aga cct cat tag caa agc aat ttt cag tta tc*	55°C	30
L. cyanellus	*Start atg gat gat gaa aty gcc gca ctg gtt* *CyanR2 tgg tta gac ctc att aga tgt cag cat atg*	55°C	30
L. gibbosus	*Lgib 318F aaa taa gta ctg tat tat agg aaa tat tac cta gac a* *Lgib 458R ccc acc atc act ccc tga aca aga cat aat*	50°C	35
L. gulosus	*Lgul 168F ata aga act tgc tga tta tgg att tta ata ctt* *Lgul 440R tca ctc cct gaa gaa gac atg aca aac ttg tta*	50°C	35
L. humilis	*Start atg gat gat gaa aty gcc gca ctg gtt* *Lhum 421R ata tgt agc agt tcc taa tta aaa aag gta ctt*	47°C	40
L. macrochirus	*Lmac 146F ata aag cca cac cgt ttt tta tgg at* *SFI2R1 aga taa ggc aca cag tca aga gag acc tgt*	50°C	35
L. marginatus	*Lmar 113F cca ggc atc agg tga gtg agt gat* *Lmar 423R gat atg tag cag ttc cta att aaa ggg aaa gag g*	53°C	35
L. megalotis	*Lmeg 396F taa tta gga act gct aca tat cat ggt gg* *SFI2R1 aga taa ggc aca cag tca aga gag acc tgt*	53°C	35
L. microlophus	*Lmic 263F agc tct aac tgc taa gca aca ttt aca acc tg* *Lmic 399R aac agt tct tca tta aag gta aag agg taa at*	53°C	35
L. miniatus	*Lmin 360F caa gtt aca tat agt cag gat ctt tat g* *Lminpun 725R gtg caa ctc tgc atg tgc aga aag ggt ac*	48°C	35
L. punctatus	*Lpun 157F gaa ctt gct gat tat gta tta ata cat* *Lpun 416R cat gga tat gta aca gtt ctt aat taa aca*	47°C	35
L. symmetricus	*Lcyasym 264F tag ctg aaa att gct ttg ctc ata tgc tga cat c* *Lsym 401R gca tgc att tgt gtc tag gca ata caa tga*	53°C	35

Figure 1. Maximum parsimony tree of *Lepomis* spp. Based on nDNA beta-actin gene sequence. Bootstrap support is shown for each branch. State localities for each sample are abbreviated.

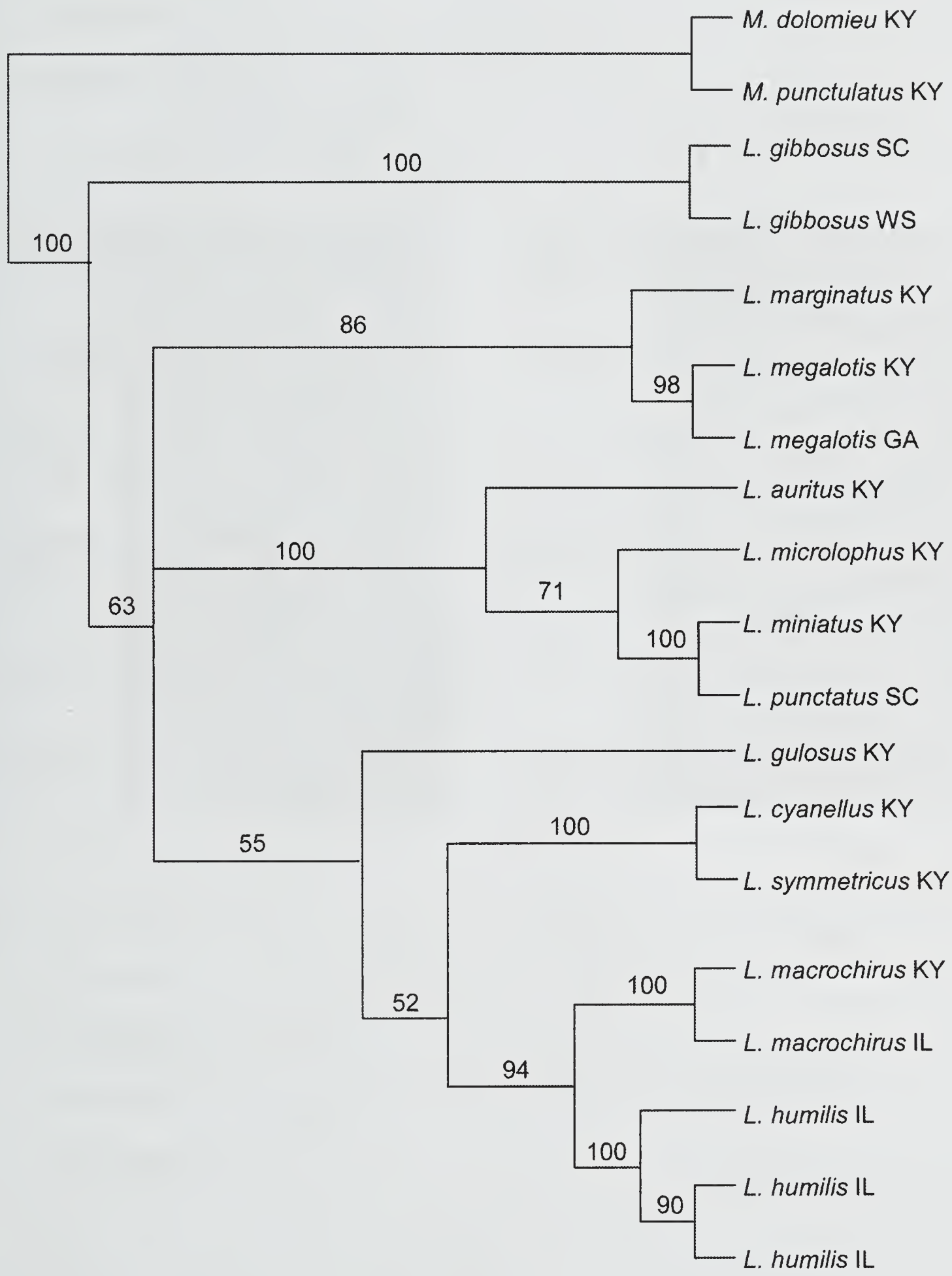


Figure 2. Maximum likelihood tree of *Lepomis* spp. Based on nDNA beta-actin gene sequence. Bootstrap support is shown for each branch. State localities for each sample are abbreviated.

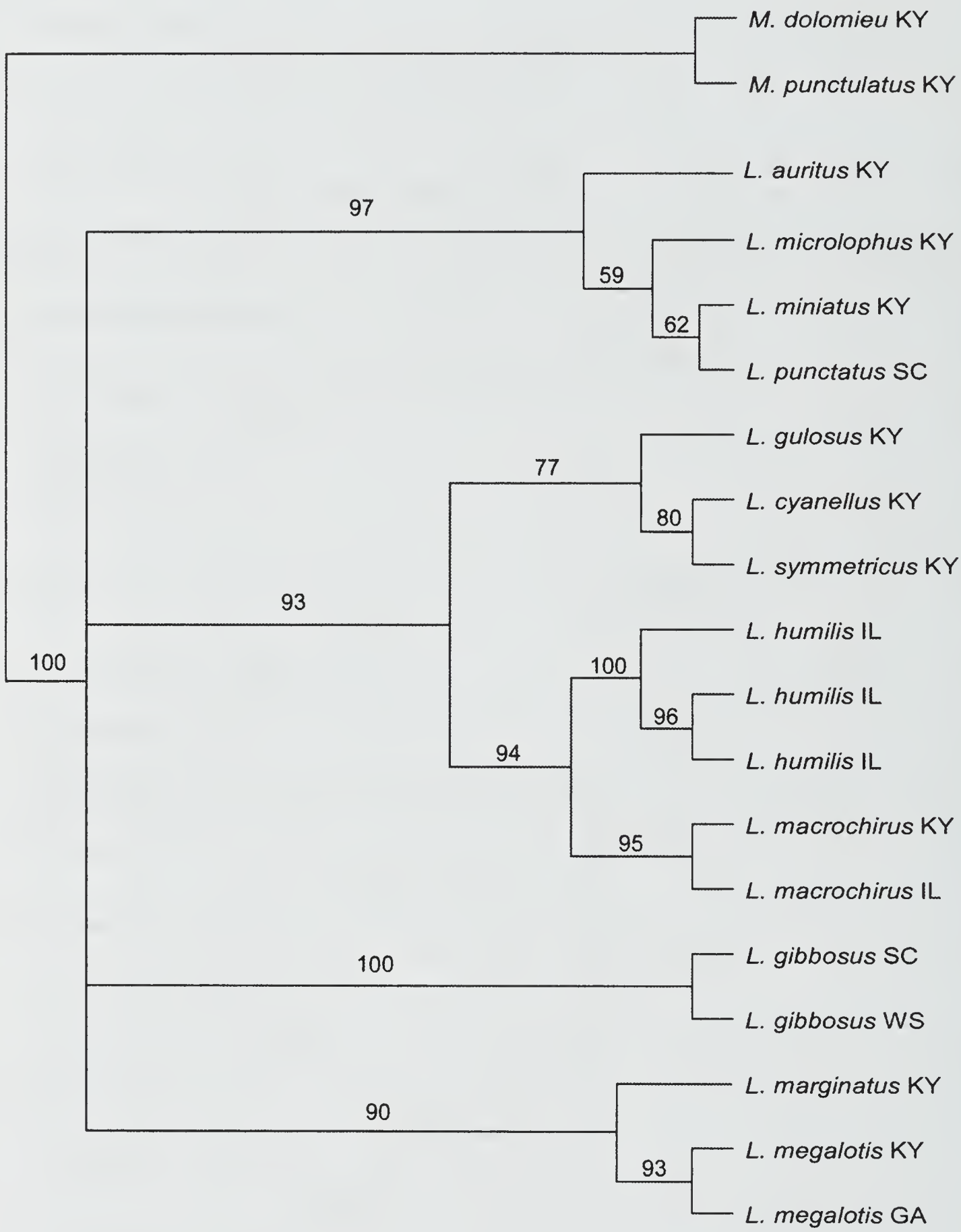
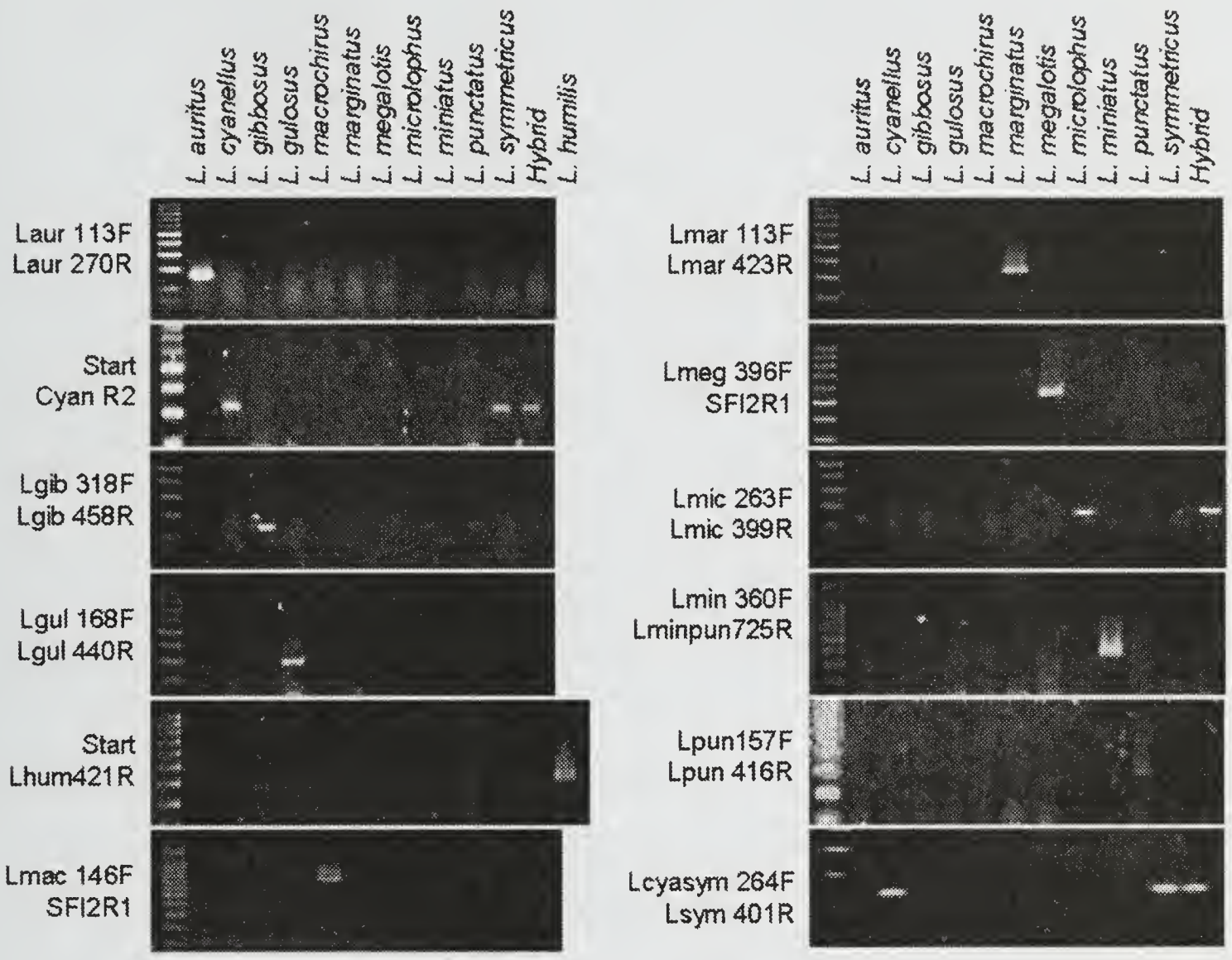


Figure 3. Products of species-specific PCR reactions to identify each of the twelve *Lepomis* species and one hybrid (*L. cyanellus* × *L. microlophus*). The first lane in each gel is a 100bp DNA ladder. The primer pair used in each case is indicated to the left of the photograph.



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Survival of Unexploited Raccoons on a Rural Refuge in Southern Illinois

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ABSTRACT

Although raccoons (*Procyon lotor*) have been studied extensively throughout their range, few studies have focused on unharvested populations in rural refuge settings. During 2003-2005, we quantified survival of unexploited raccoons on a rural refuge in southern Illinois. We captured 54 adult females and monitored them for survival via radiotelemetry for 26 months. Similar to other unexploited raccoon populations, survival was 0.80 and did not vary seasonally. Twelve of 54 radiocollared raccoons died during the study; causes of mortality were disease (n = 5), predation (n = 2), vehicle collisions (n = 1), and unknown (n = 4). Elevated survival of raccoons was indicative of highly suitable habitat and protection from harvest. Our study enhances the collective understanding of raccoon demographics and facilitates the construction of population models and management plans for unexploited raccoon populations.

Key words: demography, Illinois, *Procyon lotor*, raccoon, survival, unexploited

INTRODUCTION

Raccoon (*Procyon lotor*) populations have increased nearly 20-fold during the last 70 years (Zeveloff 2002), reaching overabundant levels in many portions of the species' geographic range. Across its range, high raccoon densities pose a threat to spread diseases such as rabies, canine distemper virus, and raccoon roundworm (*Baylisascaris procyonis*) to humans and wildlife (Mankin et al. 1999, McCleery et al. 2005, Rosatte et al. 2006, Nielsen et al. 2007). Elevated raccoon populations may also affect other wildlife species via increased predation and competition (Urban 1970, McCleery et al. 2005, Smith et al. 2006). Raccoons are also a nuisance species that frequently causes property damage (Curtis et al. 1995, Ikeda et al. 2004, Bluett 2007).

Wildlife biologists study demographic characteristics of raccoon populations to provide information for management. Most demographic studies have been conducted on harvested populations, with survival (Clark et al. 1989, Hasbrouck et al. 1992, Chamberlain et al. 1999) being one of the most researched aspects. Several investigations of unexploited raccoon populations have quantified survival rates (Riley et al. 1998, Gehrt and Fritzell 1999, Prange et al. 2003, Gehrt and Prange 2006). However, most of these studies have occurred in urban or suburban settings (Riley et al. 1998, Gehrt and Fritzell 1999, Prange et al. 2003) with one on a wildlife refuge in the Gulf Coastal Plain of Texas characterized by long hot summers and mild winters (Gehrt and Fritzell 1999).

Little information exists about raccoons in rural, protected, refuge settings that are relatively free of the human influence found in urban environments. We quantified survival of an unexploited raccoon population on a state-managed waterfowl refuge in a bottomland hardwood forest-agricultural ecosystem in the Mississippi River floodplain of southern Illinois. Our goal was to provide wildlife biologists with information useful for modeling and managing raccoon populations in floodplain ecosystems where harvest is nonexistent. We predicted that raccoon survival rates on our study area would be higher than those reported for exploited populations due to harvest protection and high-quality habitat.

MATERIALS AND METHODS

Study Area

Research was conducted on the Union County Conservation Area (UCCA), a wildlife refuge located in the Lower Mississippi River bottomlands division in southwest Illinois (UTM Zone 16 4140000N, 290000E). The UCCA encompassed 2,510 ha of which 550 ha were lakes and permanent and seasonal wetlands. Dominant aquatic vegetation in wetlands and lakes were buttonbush (*Cephalanthus occidentalis*), elodea (*Elodea spp.),* and water lily (*Nymphaea spp*.). In addition, 770 ha were bottomland hardwood forests with portions seasonally flooded. Dominant tree species included green ash (*Fraxinus pennsylvancia*), black willow (*Salix nigra*), and cottonwood (*Populus deltoides*) in wet areas, with sweetgum (*Liquidambar styraciflua*), pecan (*Carya illinoensis*), and pin oak (*Quercus palustris*) in drier areas. A total of 1190 ha consisted of agricultural fields, primarily planted in corn, soybeans, winter wheat, and milo.

Capture and Handling

Raccoon capture activities were conducted on a 197-ha portion of the UCCA. We live-captured raccoons during 4 periods: 6 October-4 December 2003, 8 March-16 April 2004, 26 September-10 December 2004, and 2 March-10 April 2005. During each trapping period, 40 wire cage traps (30x30x70 cm; Schroeder Fur Co., Grand Island, NE) were set along linear transects adjacent to water or field edges, and spaced at 100-m intervals. We baited traps with commercial cat food and checked them each morning during 0700-1100 hr.

We anesthetized captured animals with an intramuscular injection of Telazol (5mg/kg) based on an initial estimation of the animal's weight (Gehrt et al. 2001). Anesthetized animals were sexed and classified as either juveniles or adults (>1 yr) based on weight, body size, and condition of dentition (Grau et al. 1970). Mortality-sensing radiocollars (120 g; Wildlife Materials, Inc., Carbondale, Illinois, USA) were placed on selected females considered to be of an adequate size (> 3.5 kg), and all animals were ear-tagged. We released all raccoons at their capture site. Research was conducted in adherence with American Society of Mammalogists' guidelines (Animal Care and Use Committee 1998, Gannon et al. 2007) and a university-approved animal welfare protocol (Southern Illinois University Carbondale Animal Assurance #A-3078-01).

During 6 October 2003-1 November 2005, adult female raccoons were monitored for survival weekly during daytime hours (0700-1700 hr) using a TS-1 receiver and scanner (Telonics, Inc., Mesa, Arizona, USA) and a 3-element Yagi antenna. Radiocollars emitted a different signal pulse following 12 hours of inactivity; all deceased raccoons were

located by tracking these signals and mortalities were necropsied immediately following detection. We broadly classified causes of raccoon mortality into 4 categories: disease, predation, vehicle accident, and unknown. Raccoons that died within 2 weeks of collaring ($n = 3$) were not included in the analysis. Raccoons whose radiocollars failed ($n = 3$) were censored from the analysis.

Survival Analysis

We used a known fates model in program MARK (Cooch and White 2007) to estimate survival of adult female raccoons. We developed a set of 4 a priori models based on age, year, and season. We generally followed Prange et al. (2003:326-327) in separating the year into 4 seasons based on raccoon biology and general weather patterns. The pregnancy-parturition season (hereafter, spring) was during 1 March-30 May and characterized by warming weather and herbaceous and deciduous vegetation growth. The post-parturition season (hereafter, summer) was during 1 June-30 Aug and characterized by juveniles beginning to move with their mother during long, hot days and peak vegetation. The period when juveniles were weaned and began to move independently of their mother (hereafter, fall) was defined as 1 September-30 November. Leaf fall and cooling temperatures occurred during this interval. The period when raccoons were largely inactive, weather was cold, and herbaceous and deciduous vegetation reached senescence (hereafter, winter) was defined as 1 December-30 February.

Currently, there is no goodness-of-fit test for known-fate models in program MARK because each model is presumed saturated, and thus believed to fit the data perfectly (Cooch and White 2007). Therefore, we conducted a sensitivity analysis of the variance inflation factor ($\hat{c}$) by adjusting $\hat{c}$ in increments (0.25) from 1 (model fits the data) to 3 (model is overdispersed) and examined the model ranks for change (Brasher et al. 2006). If change in model order occurred after $\hat{c}$ was adjusted, we compared the model set with $\hat{c}$ set as 1 (model set 1) to the model set with the highest $\hat{c}$ (model set 2) that showed stability in the order. We tested each model independently for its influence on survival by comparing the model's ΔAIC_c to a model that held survival constant (Cooch and White 2007). We estimated survival rates and 95% confidence intervals using averages from models ≤ 2 ΔAIC_c points from the most parsimonious model (Cooch and White 2007). If 2 model sets resulted after $\hat{c}$ adjustments, we compared the estimates and 95% confidence intervals from the most parsimonious models from each model set.

RESULTS

We captured 279 different raccoons (83 adult females, 122 adult males, 35 juvenile females, and 39 juvenile males) 391 times in 6,023 trap-nights. We monitored 54 adult female raccoons for survival during 20,761 radio-days. We recorded 12 mortalities attributable to disease ($n = 5$; 42%), unknown sources ($n = 4$; 33%), predation ($n = 2$; 17%), and vehicle collisions ($n = 1$; 8%). The model order remained the same when $\hat{c}$ was adjusted, suggesting no overdispersion of the data nor a necessity for additional model sets. The most parsimonious model held survival constant and the model set provided no evidence for variation in survival by season (Table 1). However the model S(year) was approximately ≤ 2 ΔAIC_c and had a lower deviance than S(constant), offering slight evidence for an annual effect on survival (Table 1). Based on the most parsimonious model, annual survival was 0.80 (95% CI=0.70-0.90).

Table 1. Survival models for unexploited raccoons in southern Illinois, USA, 2003-2005.

Model	AIC_c [a]	ΔAIC_c	w_i [b]	K [c]	Deviance
S(constant)	121.56	0.00	0.71	1	21.07
S(year)	123.56	2.01	0.26	3	19.05
S(season)	127.59	6.04	0.03	4	21.06
S(season x year)	133.17	11.62	0.00	9	16.42

[a] Models were ranked using Akaike's Information Criterion corrected for small sample size (AIC_c).
[b] Akaike model weight.
[c] No. of parameters estimated.

DISCUSSION

Harvest is commonly the most significant mortality factor in exploited raccoon populations (Sanderson 1961, Johnson 1970), accounting for ≥78% of raccoon deaths (Clark et al. 1989). However, in unexploited raccoon populations, disease is generally the most common cause of mortality (Gehrt et al. 1990, Roscoe 1993, Riley et al. 1998, Gehrt and Fritzell 1999). We found a similar percentage of raccoons dying from disease as other studies of unexploited raccoons and observed a comparable percentage of vehicle-collision mortalities to harvested populations studied by Clark et al. (10%; 1989) and Hasbrouck et al. (6%; 1992).

In the absence of anthropogenic factors, natural mortality agents have little effect on most raccoon populations (Johnson 1970, Gehrt and Fritzell 1999), thus protected raccoon populations tend to have higher survival rates than exploited populations. Within the literature, there is a wide range of adult female survival rates reported for unexploited raccoon populations (0.57 – 0.88), and estimates derived in our study fell within that range (Table 2). As expected, adult female survival on the UCCA was higher than those adult survival rates presented in studies of harvested raccoon populations (Table 2). Compared to harvested populations that combined sexes and age classes for analysis, female raccoon survival at UCCA appeared higher than Mankin et al. (1999) and Pitt et al. (2004) (Table 2).

Similar to other studies of unexploited populations (Riley et al. 1998, Gehrt and Fritzell 1999, Prange et al. 2003), we found no seasonal differences in raccoon survival. These results were unsurprising given that most seasonal differences in survival can be attributed to harvest (Clark et al. 1989, Brown et al. 1990, Hasbrouck et al. 1992). The slight evidence for a year effect on survival was likely attributable to natural fluctuations in annual survival rates observed by other researchers (Hasbrouck et al. 1992, Gerht and Fritzell 1999, Prange et al. 2003).

Table 2. Selected annual survival estimates for raccoons across the species' range, 1989-2008.

Source (state/province)	Adult survival[a]	Harvested/ protected	Site description
Clark et al. (1989), IA	0.47-0.75	Harvested	Agriculture
Brown et al. (1990), PA	0.67-0.69	Harvested	Forested game lands
Hasbrouck et al. (1992), IA	0.53	Harvested	Agriculture
Riley et al. (1998), DC	0.71-0.73	Protected	Urban deciduous forest
Chamberlain et al. (1999), MS	0.50[b]	Harvested	Bottomland hardwood, mixed pine-hardwood forests
Gehrt and Fritzell (1999), TX	0.84	Protected	Gulf plains, prairies, marshes
Mankin et al. (1999), IL	0.74[c]	Harvested	Agriculture, pasture, shrub, forest
Mosillo et al. (1999), IL	0.70-0.73	Protected	Mature upland forest, old field, mesic grassland
Prange et al. (2003), IL	0.63-0.88[b]	Protected	Commercial, industry
	0.57-0.74[b]	Protected	Forest, agriculture, grassland
	0.59-0.80[b]	Protected	Wetland, grassland, savanna
Pitt et al. (2008), Saskatchewan	0.51-0.84[c]	Harvested	Agriculture
This study, IL	0.80[b]	Protected	Bottomland hardwood forest

[a] Survival rates are for both sexes combined, unless otherwise noted.
[b] Adult female survival rate.
[c] Study combined both sexes and age classes into one survival rate.

Our survival estimate was higher than the only other study found in the literature that examined survival of adult female raccoons in a bottomland hardwood forest environment (Chamberlain et al. 1999; Table 2). Chamberlain et al. (1999) also noted a significantly lower overall survival estimate during their breeding-gestation (1 Feb-31 May) period (0.65) compared to their parturition-young rearing (1 Jun-30 Sep) period (0.93), which was most likely due to harvest. After an exploratory analysis adjusting our data to Chamberlain's seasons, we still could conclude no seasonal differences in adult female survival.

We found only 3 records of survival estimates of Illinois raccoons based on radiotelemetry data: Mankin et al. (1999), Mosillo et al. (1999), and Prange et al. (2003). Our survival estimate fell within the ranges reported by both Mankin et al. (1999) and Prange et al. (2003), even though the population Mankin et al. (1999) studied was harvested. Unlike many harvested populations studied, Mankin et al. (1999) observed no notable increase in juveniles harvested in comparison to adults which may account for the similar survival estimates to protected raccoon studies. Our survival estimate was higher than those presented by Mosillo et al. (1999), which may be a result of better habitat. Raccoons were studied by Mosillo et al. (1999) in to a forest preserve dominated by a mature upland forest while our raccoons resided in mature bottomland hardwood forest with ample wetlands.

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LITERATURE CITED

Bluett, B. 2007. Wildlife damage management in Illinois—2006 summary. Illinois Department of Natural Resources, Wildlife Diversity Program, Springfield, Illinois, USA.

Brasher, M. G., T. W. Arnold, J. H. Devries, and R. M. Kaminski. 2006. Breeding-season survival of male and female mallards in Canada's prairie-parklands. Journal of Wildlife Management 70:805-811.

Brown, C. L., C. E. Rupprecht, and W. M. Tzilkowski. 1990. Adult raccoon survival in an enzootic rabies area of Pennsylvania. Journal of Wildlife Diseases 26:346-350.

Chamberlain, M. J., K. M. Hodges, B. D. Leopold, and T. S. Wilson. 1999. Survival and cause-specific mortality of adult raccoons in central Mississippi. Journal of Wildlife Management 63:880-888.

Clark, W. R., J. J. Hasbrouck, J. M. Kienzler, and T. F. Glueck. 1989. Vital statistics and harvest of an Iowa raccoon population. Journal of Wildlife Management 53:982-990.

Cooch, E., and G. White. 2007. Program MARK: a gentle introduction. Sixth edition. Fort Collins, Colorado, USA.

Curtis, P. D., M. E. Richmond, P. A. Wellner, and B. Tullar. 1995. Characteristics of the private nuisance wildlife control industry in New York. Proceedings from the Eastern Wildlife Damage Control Conference 6:49-57.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild animals in research. Journal of Mammalogy 88:809-823.

Gehrt, S. D., D. L. Spencer, and L. B. Fox. 1990. Raccoon denning behavior in eastern Kansas as determined from radio-telemetry. Translations of the Kansas Academy of Science 93:71-78.

Gehrt, S. D., and E. K. Fritzell. 1999. Survivorship of a nonharvested raccoon population in south Texas. Journal of Wildlife Management 63:889-894.

Gehrt, S. D., L. L. Hungerford, and S. Hatten. 2001. Drug effects on recaptures of raccoons. Wildlife Society Bulletin 29:833-837.

Gehrt, S. D., and S. Prange. 2006. Interference competition between coyotes and raccoons: a test of the mesopredator release hypothesis. Behavioral Ecology 18:204-214.

Grau, G. A., G. C. Sanderson, and J. P. Rogers. 1970. Age determination of raccoons. Journal of Wildlife Management 34:364-372.

Hasbrouck, J. J., W. R. Clark, and R. D. Andrews. 1992. Factors associated with raccoon mortality in Iowa. Journal of Wildlife Management 56:693-699.

Ikeda, T., M. Asano, Y. Matoba, and G. Abe. 2004. Present status of invasive alien raccoon and its impact in Japan. Global Environmental Research 8:125-131.

Johnson, A. S. 1970. Biology of the raccoon (*Procyon lotor varius Nelson and Goldman*) in Alabama. Agricultural Experiment Station, Auburn University Bulletin 402, Auburn, Alabama, USA.

Mankin, Ph. C., C. M. Nixon, J. B. Sullivan, T. L. Esker, R. G. Koerkenmeier, and L. L. Hugerford. 1999. Raccoon (*Procyon lotor*) survival in west-central Illinois. Transactions of the Illinois State Academy of Science 92:247-256.

McCleery, R. A., G. W. Foster, R. R. Lopez, M. J. Peterson, D. J. Forrester, and N. J. Silvy. 2005. Survey of Raccoons on Key Largo, Florida, USA, for *Baylisascaris procyonis*. Journal of Wildlife Diseases 41:250-252.

Mosillo, M. E., E. J. Heske, and J. D. Thompson. 1999. Survival and movements of translocated raccoons in northcentral Illinois. Journal of Wildlife Management 63:278-286.

Nielsen, C. K., A. Defore, and E. Bade. 2007. Survey of *Baylisascaris procyonis* and canine distemper virus in southern Illinois raccoons. Transactions of the Illinois State Academy of Science 100:169-176.

Pitt, J. A., S. Lariviere, and F. Messier. 2008. Survival and body condition of raccoons at the edge of the range. Journal of Wildlife Management 72:389-395.

Prange, S., S. D. Gehrt, and E. P. Wiggers. 2003. Demographic factors contributing to high raccoon densities in urban landscapes. Journal of Wildlife Management 67:324-333.

Riley, S. P. D., J. Hadidian, and D. A. Manski. 1998. Population density, survival, and rabies in raccoons in an urban national park. Canadian Journal of Zoology 76:1153-1164.

Rosatte, R., K. Sobey, D. Donovan, M. Allan, L. Bruce, T. Buchanan, and C. Davies. 2006. Raccoon density and movements after population reduction to control rabies. Journal of Wildlife Management 71:2373-2378.

Roscoe, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. Journal of Wildlife Diseases 29:390-395.

Sanderson, G. C. 1961. Techniques for determining age of raccoons. Illinois Department of Natural Resources, Illinois Natural History Survey 45:1-16, Urbana, Illinois, USA.

Smith, H. T., W. E. Meshaka, Jr., R. M. Engeman, S. M. Crossett, M. E. Foley, and G. Bush. 2006. Raccoon predation as a potential limiting factor in the success of the green iguana in southern Florida. Journal of Kansas Herpetology 20:7-8.

Urban, D. 1970. Raccoon populations, movement patterns, and predation on a managed waterfowl marsh. Journal of Wildlife Management 34:372-282.

Zeveloff, S. I. 2002. Raccoons: a natural history. Smithsonian Institute Press, Washington, D. C., USA.

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Annual Occurrence and Abundance of Araneae Families in a Tall Grass Prairie in Illinois Before and After a Controlled Prairie Burn

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ABSTRACT

We examined how simulated natural disasters affect spider populations on a conserved, re-planted prairie at Rock Springs Center for Environmental Discovery in Macon Co., Decatur, Il. We created 15 plots evenly spaced on the prairie and sampled them by sweep netting from 1 June 2005 - 12 October 2006. This allowed us to obtain 10 months of data before the prairie burned on 9 March 2006 and 7 months of data afterwards. A Simpson Index found the spider community more diverse after the burn than before. A t-test also determined that there was a significant decrease in the families Salticidae and Araneidae between the pre-burn and post-burn periods. Although there was no statistical difference in the number of tetragnathids in the pre-burn period versus the post-burn period, we did note that the number of pisaurids increased at the same time the tetragnathid numbers decreased, indicating a possible interaction between these two families.

INTRODUCTION

Modern conservation planning increasingly relies on the use of ecological restoration techniques to simulate previous communities. Many studies in these sites focus only on the vegetation while the rest of the ecosystem is overlooked. Studying arthropods in these areas is beneficial because arthropods have short generation times, small size, diversity of microhabitats and niches, and play very important ecological roles (Longcore, 2003). Additionally, their large population sizes allow for statistically significant analysis. Therefore, arthropods have been used to track ecological changes in a multitude of studies (Niwa and Peck, 2002).

Simulated wildfires are a popular management practice in many conservation areas. Controlled wildfires clear much of the dead plant matter that could cause a devastating fire and are thought to help to create a higher diversity level. However, this diversity hypothesis has not been adequately tested with spiders, because few spider surveys have been conducted before and after a simulated disturbance. Evidence suggests that the number and identity of species in an ecosystem correlates directly to the stability and resilience of the ecosystem itself (Buddle et al., 2006). We hypothesized that the spider diversity after a prairie burn at the Rock Springs Center for Environmental Discovery

would increase as has been indicated for other species of spiders after a wetland burn (Johnson, 1995).

Survey studies are reliable tools when assessing conservation priorities because they can provide an indication of diversity level and can be used to help assess how human influence affects the diversity of an area (Cardoso et al., 2004). It is important to keep high priority areas conserved and protected from human influences, such as residential development and pesticides, in order to maintain a high diversity level. Areas with increased human influence show less diversity than areas with less human influence (Cardoso et al., 2004).

We conducted this study to examine how a simulated natural disaster affects the spider populations on a conserved prairie. We sampled the prairie for 10 months prior to a simulated burn to collect baseline data. We then sampled for 7 months after the prairie was burned to examine spider family succession. The data were analyzed to determine whether there was a significant difference between the pre-burn and post-burn spider community in number and types of spider families present.

MATERIALS AND METHODS

Our study was conducted at the Rock Springs Center for Environmental Discovery in Macon Co., Decatur, Il. Five sample plots on three transects (Fig. 1) were sampled weekly from 1 June 2005 until the prairie experienced its first frost on 15 November 2005. After the first frost, we sampled biweekly. We resumed sampling weekly when the Macon County Conservation District burned the prairie on 9 March 2006 and sampled weekly until the first frost on 12 October 2006; the prairie was also checked after this date to verify that spiders were no longer on vegetation.

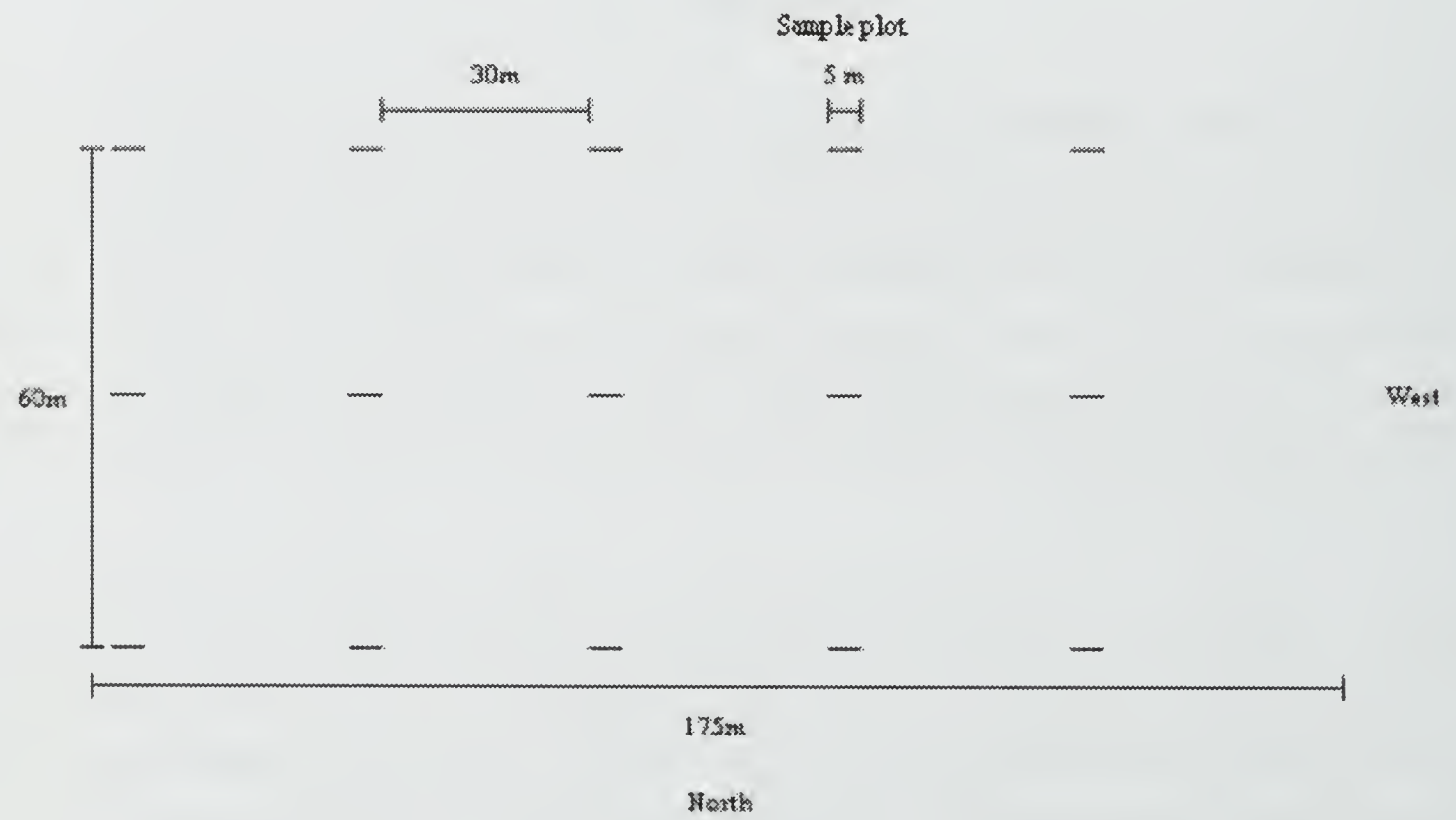

Figure 1. Rock Springs Center for Environmental Discovery study site divided into 15 sampling plots (5.0 m) in 3 transects with 30 m between each plot.

We used sweep net sampling to collect spiders in each plot. For each of the 15 plots, we began sampling at the beginning of the plot and performed 10 sweeps on both sides of the plot to ensure that the plot was evenly sampled. After 20 sweeps in each plot, we sorted the net litter, removed all spiders, and preserved them in 80% ethanol.

While there are a variety of sampling options, we chose sweep net sampling for several reasons. First, sweep net sampling collections represent a large variety of the total flora because different heights can be swept (Standen, 2000). In addition, this sampling method did not cause extreme damage or disturbance to the prairie. Pitfall traps (another frequently used sampling method) can damage the environment and can have misleading results due to grass density. We performed a large number of sweeps per plot (n = 20) and sampled over a lengthy time period to ensure effectiveness in capturing spiders despite heterogeneity of habitat. Pitfall traps are also limited to ground-dwelling spiders and exclude many web-building spiders and spiders that dwell on higher foliage (Johnson, 1996), while sweep net sampling covers a sufficient degree of habitat stratum (Dobyns, 1997). It is important to collect foliage dwellers because spiders in higher strata might play a more important role in biological control than those of other strata (Ludy and Lang, 2004).

After sampling, we transferred the spiders to Millikin University, where we identified each spider to the family level following Roth (1985). After our 1.5 years of sampling, we analyzed the data using a two sample t-test assuming unequal variances to compare the number of individuals within each family from the pre-burn versus post-burn community (Press, 1992). We performed t-test analyses on the data collected from June - October to ensure consistency between each period.

We used a Simpson Index (Simpson, 1949) to determine the level of species diversity in the pre-burn and post-burn community. The Simpson Index is used to measure diversity and is often used to quantify biodiversity in ecological habitats. This index focuses on the number of species as well as the abundance of each species, and represents the probability that two randomly selected individuals of a given habitat belong to the same species.

RESULTS

The number of individual spiders was separated by month collected and family for the pre-burn and post-burn years (Tables 1 and 2). The number that was collected each month was then divided by the number of collections for that month to provide consistency since some months had more sampling periods than others.

There was a significant decrease in the number of individuals in the families Salticidae (Fig. 2) and Araneidae (Fig. 3) from pre-burn to post-burn period ($P < 0.01$). The Simpson Index value (D = 0.36) obtained for the pre-burn community indicates a moderate level of spider family diversity. For the post-burn community the Simpson Index (D = 0.26) indicates greater diversity (9 families collected) when compared to the pre-burn community (6 families collected) (Tables 1 and 2).

Even though there was no statistical difference from the pre-burn to post-burn period in tetragnathids, there were no tetragnathids in the pre-burn period and 43 in the post-burn period. There was also an increase in the number of pisaurids as tetragnathid numbers began to decline (Table 2 and Fig. 4).

Table 1. Spiders collected in the pre-burn period separated by month and family from June – October 2005. Spiders were not present from November 2005 – March 2006. The prairie was burned on 9 March 2006.

Pre-burn

	Jun	Jul	Aug	Sep	Oct	**Total**
Salticidae	20	37	39	30	7	133
Pisauridae	7	6	5	11	21	50
Thomisidae	4	1	2	1	0	8
Clubionidae	7	0	1	2	0	10
Araneidae	7	8	3	12	5	35
Oxyopidae	1	6	1	0	0	8

Table 2. Spiders collected in the post-burn period separated by month and family from April – October 2006. Spiders were not present from November 2005 – March 2006. The prairie was burned on 9 March 2006.

Post-burn

	Apr	May	Jun	Jul	Aug	Sep	Oct	**Total**
Salticidae	2	2	5	15	7	7	1	39
Pisauridae		1	4	5	7	12	3	32
Oxyopidae			1	2		4		7
Tetragnathidae				25	17	1		43
Lycosidae				1		1		2
Thomisidae				1		1		2
Theridiidae						1		1
Linyphiidae				2				2
Araneidae						1		1

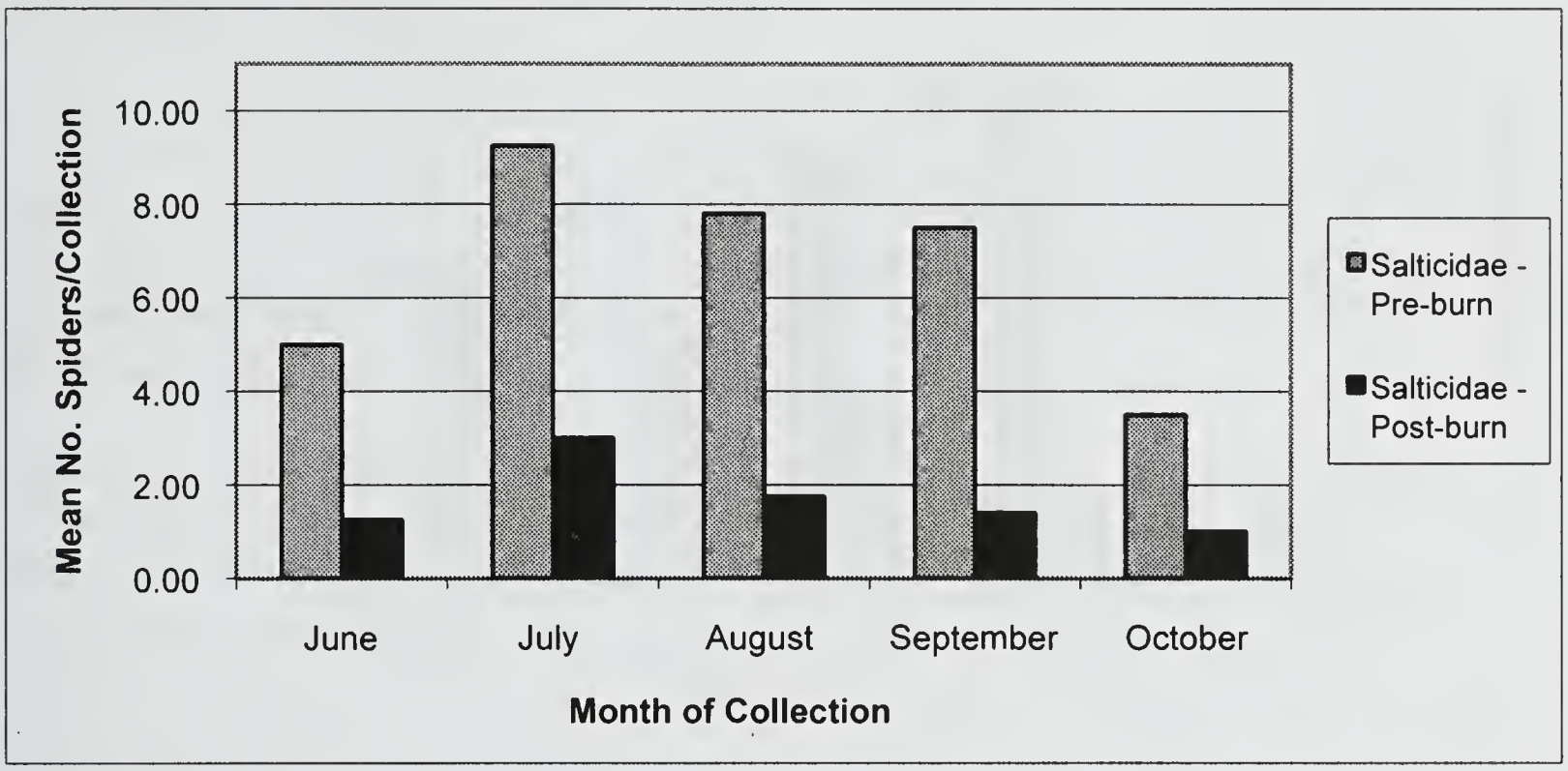

Figure 2. The mean number of salticids per collection between the pre-burn and post-burn periods separated by month. A two-tailed t-test assuming unequal variances showed a significant decrease in numbers after the burn ($t = 2.7$, $df = 5$, $P < 0.01$).

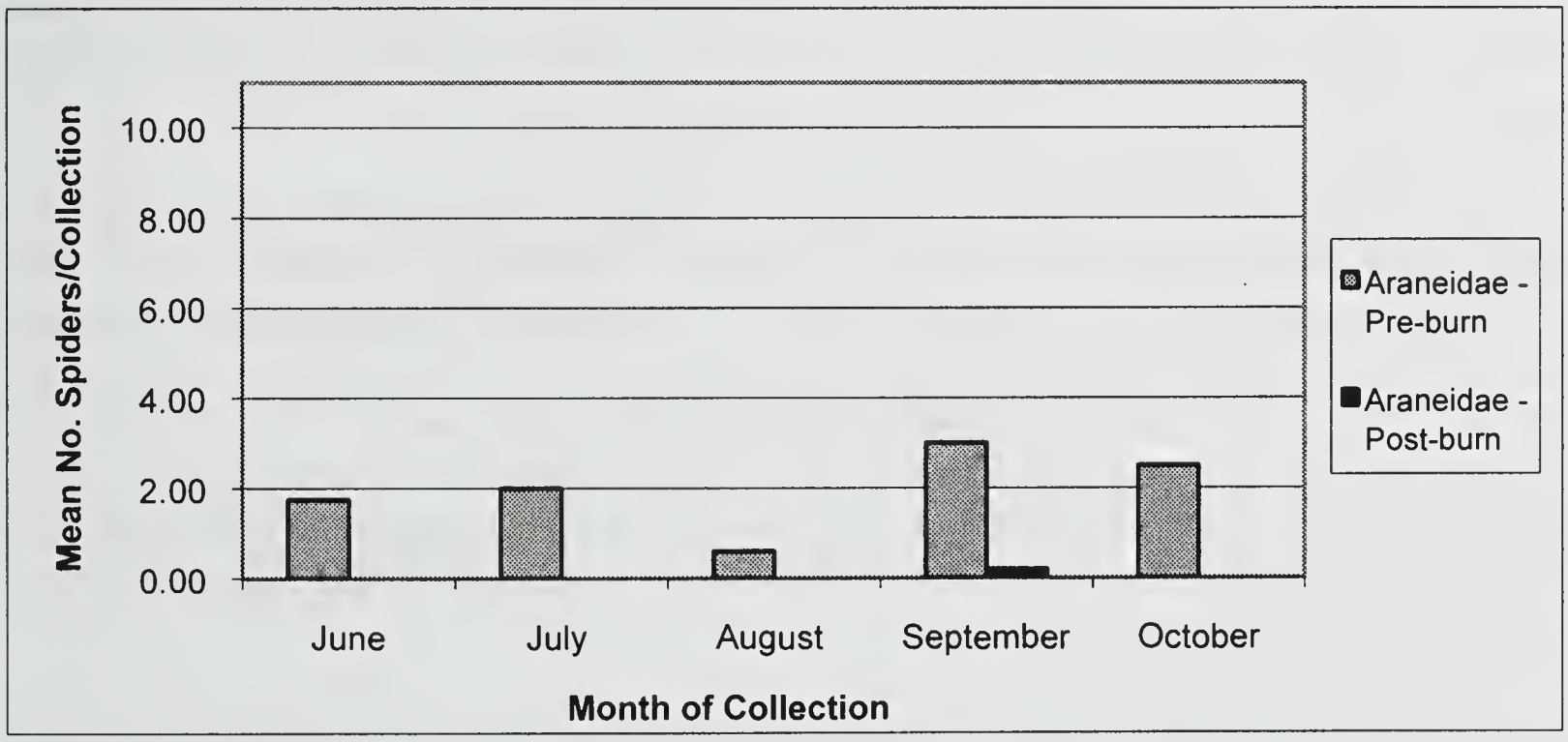

Figure 3. The mean number of araneids per collection between the pre-burn and post burn periods separated by month. A two-tailed t-test assuming unequal variances showed a significant decrease in numbers after the burn ($t = 2.7$, $df = 4$, $P < 0.01$).

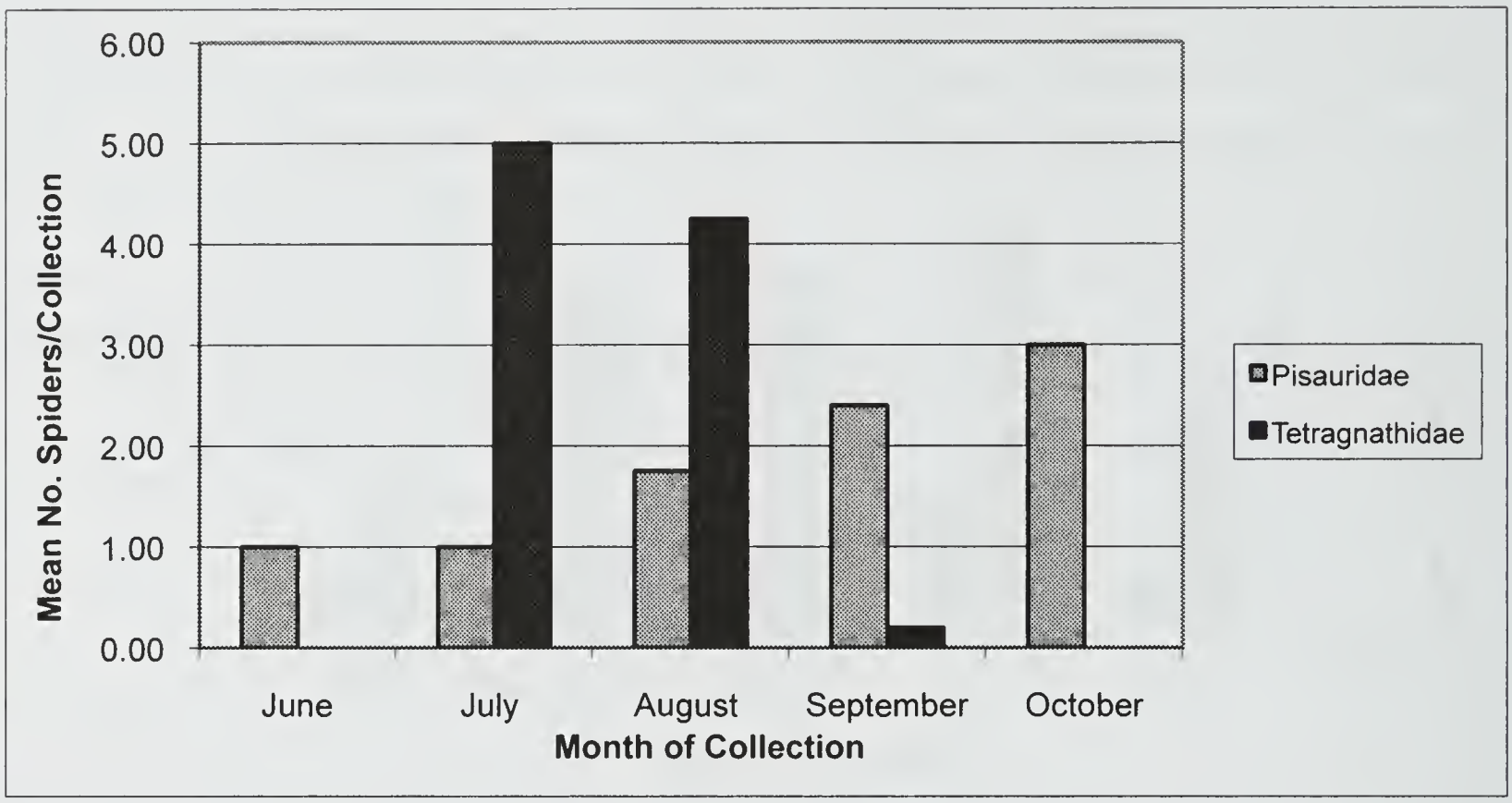

Figure 4. The mean number of pisaurids and tetragnathids per collection in the post-burn period separated by month.

DISCUSSION

During the pre-burn period, we collected 244 spiders, while in the post-burn period, we collected 129 spiders. A higher abundance of arthropods in an indigenous prairie, compared to that of one with a controlled burn, was also shown by McIntire and Thomson (2002).

There was a significant decrease in the number of salticids and araneids present after the prairie burn. In the pre-burn period, both vegetation and the insect populations were likely more stable and able to support the carnivorous spiders. This may have affected the salticids in particular because in the pre-burn period, we noted an abundance of salticids earlier in the season, starting in July, and spiders active in the early summer are affected by fires because the indigenous plants have not had sufficient time to renew growth (Johnson, 1995). Salticids reach their maturity and breeding season in earlier summer months, having larger numbers in July, while many other families reach maturity in September and October.

There were differences in family diversity between the two years. The Simpson Index for the post-burn year was lower than for the pre-burn year, indicating a greater diversity after the burn than before. The family Clubionidae was the only family found before, but not after the prairie burn. However, after the burn, we collected four families (Tetragnathidae, Lycosidae, Theridiidae, and Linyphiidae) not found in the pre-burn community. It is plausible that when the prairie was burned, it destroyed some families that had monopolized resources before and thus hindered other families from becoming established. When new vegetation emerged, each family had an opportunity to colonize it, leading to greater diversity, as observed in a forest system after a burn (Buddle et al., 2006). Additionally, when a hurricane destroyed the spider community on a small island,

a similar trend of a decrease in the number of spiders, but an increase in species diversity was observed (Schoener and Spiller, 2005).

With tetragnathids, we did not note any statistical difference in numbers from pre-burn to post-burn; however, we do feel the differences are notable because we observed a decrease in tetragnathids as pisaurids increased after the burn. This could be because as the pisaurids reached maturity and bred in the later months, they preyed on tetragnathids, in addition to insects. This could also explain the absence of tetragnathids in the pre-burn year.

Dramatic changes in prairies have been shown to have effects on multiple trophic levels. When plants (primary producers) are destroyed, it affects primary consumers. This in turn affects the secondary and tertiary consumers, the focus of our study. While the community from the pre-burn year was also a result of secondary succession, we still noted differences between the community before the burn versus after the burn. This may be because as previously dominant families were destroyed, it enabled other families to become established in the new environment.

Succession has dramatic implications in terms of community composition, and it is important to understand the effects of human disturbances on established communities. More research with different habitats and organisms is necessary to determine the impacts of simulated wildfires and human influence on community composition.

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LITERATURE CITED

Buddle, C. M., D. W. Langor, G. R. Pohl, and J. R. Spence. 2006. Arthropod responses to harvesting and wildfire: Implications for emulation of natural disturbance in forest management. Biological Conservation. 128:346-57.

Cardoso, P., I. Silva, N.G. de Oliveira, and A. R. M. Serrano. 2004. Indicator taxa of spider (Araneae) diversity and their efficiency in conservation. Biological Conservation. 120: 517-524.

Dobyns, J. R. 1997. Effects of sampling intensity on the collection of spider (Araneae) species and the estimation of species richness. Environmental Entomology. 26(2):150-62.

Johnson, S. R. 1995. Spider communities in the canopies of annually burned and long-term unburned *Spartina pectina* wetlands. Environmental Entomology. 24(4): 832-4.

Johnson, S. R. 1996. Use of Coleopteran prey by *Phidippus audax* (Araneae, Salticidae) in tallgrass prairie wetlands. Journal of Arachnology. 24:39-42.

Longcore, T. 2003. Terrestrial arthropods as indicators of ecological restoration success in costal sage scrub. Restoration Ecology. 11: 397-409.

Ludy, C. and A. Lang. 2004. How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins: a comparison of two sampling methods. Journal of Applied Entomology. 102: 219-231.

McIntire, N. E., T. R. Thompson. 2002. A comparison of conservation reserve program habitat plantings with respect to arthropod prey for grassland birds. The American Midland Naturalist. 150: 291-301.

Niwa, C. G., R. W. Peck. 2002. Influence of prescribed fire on carabid beetle and spider assemblages in forest litter in Southwest Oregon. Environmental Entomology. 31 (5): 785-96.

Press, W. A. 1992. Numerical Recipes in C: The Art of Scientific Computing. Cambridge University Press. 616.

Roth, V. D. 1985. Spider Genera of North America. American Arachnology Society. University of Florida, Gainsville.

Schoener, T. W., D. A. Spiller. 2005. Nonsynchronous recovery of community characteristics in island spiders after a catastrophic hurricane. Proceedings of the National Academy of Sciences. 103: 2220-5.

Simpson, E. 1949. Measurement of Diversity. Nature. 163:688.

Standen, V. 2000. The adequacy of collecting techniques for estimating species richness of grassland invertebrates. Journal of Applied Ecology. 37: 884-93.

102nd Annual Meeting
Illinois State Academy of Science

April 9-10, 2009
Millikin University

KEYNOTE ADDRESS

Future of Agriculture as a Renewable Resource

Mark Matlock
Senior Vice President of Research
Archer Daniels Midland

TENTATIVE SCHEDULE

Friday, April 9th

1:00pm – 3:00pm	Graduate Student & Job Fair
3:15pm – 5:15pm	Poster Session
5:30pm – 7:30pm	Banquet & Keynote Address
7:45pm – 9:15pm	Facilitated Star Gazing

Saturday, April 10th

7:30am – 8:00am	Continental Breakfast
8:00am – 12noon	Oral Presentations & Division Meetings
12noon – 1:30pm	Lunch & ISAS Business Meeting
1:30pm – 4:30pm	Oral Presentations & Division Meetings
4:30pm – 5:00pm	Award Announcements

2009 Annual Meeting Student Awards

BOTANY DIVISION

Oral Presentation Awards

1st Place – **Katherine Chi**, University of Illinois – Urbana-Champaign
Population Surveys and Reproductive Ecology of *Synthris bullii* (Plantaginaceae), a Rare Illinois Species

2nd Place – **Craig R. McLane**, Southern Illinois University – Carbondale
Evaluating Exotic Species Assemblages Across a Chronosequence of Restored Floodplain Forests

Poster Presentation Awards

1st Place – **Zachary L. French**, Southern Illinois University – Edwardsville
Quantitative Assessment of Edge Effects in Oak-Hickory Forest Fragments in Southwestern Illinois

2nd Place – **Brad M. Daugherty**, Eastern Illinois University
Influence of Liquid Hickory Smoke Seasoning on Seed Germination of Twenty Prairie Plant Species

2009 Annual Meeting Student Awards – cont'd

CELLULAR, MOLECULAR, AND DEVELOPMENTAL BIOLOGY DIVISION

Oral Presentation Awards

Graduate – Sharon Hickman, Eastern Illinois University
Genome Wide Analysis of Gene Silencing in Mammalian Cell Hybrids

Undergraduate – Jamie N. Hadac, Knox College
Characterization of Nuclear Localization of Intersectin During *Xenopus laevis* (Anura Pipidae) Development

Undergraduate – Megan M. Brady, Knox College
Upregulation of Telomerase During Regeneration in *Dugesia tigrina* (Tubellaria)

Poster Presentation Awards

Outstanding – Joshua D. Kinser, Southern Illinois University – Edwardsville
Basic Characterization of Gravity Persistent Signal 5 (GPS5), a Hyper-Gravitropic Mutant in *Arabidopsis thaliana*

Meritorious – Jordon C. Angle, Eastern Illinois University
Analysis of Transcription Factor Promoter Binding in Rat Hepatoma/Fiboroblast Hybrids

Meritorious – Michele C. Darrow, Knox College
Characterization of Acute Ethanol Exposure on LPS-Induced Signaling Pathways in RAW 264.7 Macrophages

2009 Annual Meeting Student Awards – cont'd

CHEMISTRY DIVISION

Oral Presentation Award

Nicholas Van Hise, Bradley University

Synthesis and Antibiotic Testing of 5-aryl-3-oxo-δ-lactones

Poster Presentation Awards

1st Place (tie) – **Kelli A. Levek**, Southern Illinois University – Edwardsville

Chemical and Thermal Stability of the Calcium Binding Domain of NADPH Oxidase 5

1st Place (tie) – **Romina J. Pagliero**, Universidad Nacional de Córdoba, Argentina

Solution-Phase Parallel Synthesis of N-benzenesulfonyl Derivatives of Hetrocyles

COMPUTER SCIENCE DIVISION

Oral Presentation Award

Raaj Remesh, Western Illinois University

HTTPA

ENVIRONMENTAL SCIENCE DIVISION

Oral Presentation Award

Clint S. Moore, Knox College

Ecological Census of Reef Habitats Adjacent to Tobacco Caye, Belize C.A.

Poster Presentation Award

Eric A. Scholl, Millikin University

The Effects of Agriculture and Urbanization on Water Quality of Stevens Creek in Macon County, Illinois

2009 Annual Meeting Student Awards – cont'd

HEALTH SCIENCES DIVISION

Oral Presentation Award

Sarah M. West, Knox College

A Longitudinal Study Investigating the Forces Impacting Articular Cartilage in the Knees of Football Players

Poster Presentation Award

Kathleen A. Beeson, Knox College

The Effect of Atrazine on Learning and Memory in Young Sprague-Dawley Rats

MICROBIOLOGY DIVISION

Oral Presentation Award

Oliwia Zurek, Knox College

A Simulated Digestion of Echinacea Induces Macrophages to Secrete Pro- and Anti-Inflammatory Cytokines Through Multiple Receptors

Poster Presentation Award

Cory D. Penn, Eastern Illinois University

Degradation of Salicylate, an Important Plant Signaling Molecule, by the Fungal Plant Pathogen *Sclerotinia sclerotiorum* (order: Helotiales and family: Sclerotiniaceae)

2009 Annual Meeting Student Awards – cont'd

ZOOLOGY DIVISION

Oral Presentation Awards

Graduate – Shannon L. Fredebaugh, University of Illinois – Urbana-Champaign

Cats (Felidae) and the Risk of *Toxoplasma gondii* Infection in Terrestrial Wildlife: A Measure of Ecosystem Health

Undergraduate – Paul W. Hamilton, Illinois College

Color Preferences of Day-Flying Insects Inhabiting Disturbed and Undisturbed Sites Near Monteverde Cloud Forest Preserve, Costa Rica

Poster Presentation Award

Morgan L Holmes, Millikin University

Operant Conditioning Via Electrical Stimulus in Millipedes *Orthoporus texicolens* (Diplopoda:Spirostreptida)

2009 Student Research Awards

Mathew Bartucci, Loyola University

Sheila Langosch, University of Illinois – Chicago

David Zaya, University of Illinois – Chicago

Shannon Fredebaugh, University of Illinois – Urbana-Champaign

Nathan Fritz, Charleston High School

Robert Schorsch, Southern Illinois University – Edwardsville

Jenna McAdoo, Northern Illinois University

2009 Student Botany Travel Grant Recipient

Melissa Simpson, Southern Illinois University – Carbondale

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INFORMATION FOR CONTRIBUTORS

Submission Information

1. Send original communications to: Dr. Teresa North, *Transactions* Editor; 1713 W. Adams Rd.; Macomb IL 61455.
2. Submission of a manuscript to the Editor will be held to imply that it has not been previously published except in Abstract form and, if accepted, will not be published elsewhere in the same form without written consent of the Editor.
3. The format for manuscripts should follow a form appropriate to the specific discipline: most commonly, e.g., Abstract (no more than 250 words), Introduction, Materials and Methods, Results, Discussion, and Summary.
4. Manuscripts must be typewritten, double-spaced on one side of bond paper (8.5"x11") with all margins of not less than 1.0 inch. The author must submit one original and two copies, and should retain a copy.
5. Tables and figures may be included at the end of the manuscript or incorporated within the body of the manuscript. Tables must be numbered consecutively in the order in which they are referred to in the text. Likewise for figures.
6. Genus and species must be given for all organisms used in the investigation. Common names are also recommended.
7. In the text, references should be quoted by the author's name and date, e.g., (Smith, 1960). Footnotes are to be avoided.
8. At the end of the manuscript, full references, must be listed in alphabetical order and include the names of the authors, date of publication, full title of the paper, title of the journal, volume number, and first and last page numbers. References to books must include the number of the edition, publisher, and place of publication. Online references must include appropriate URL.

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